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Incidence of Multi-Drug Resistant (MDR) Organisms in Some Poultry Feeds Sold in Calabar Metropolis, Nigeria

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Abstract: This study reports on the incidence of Multi-Drug Resistant (MDR) organisms in poultry feeds sold in Calabar metropolis, Nigeria. Twenty samples of poultry feeds were purchased from different locations and analyzed microbiologically using standard methods. Significant enough to note is the microbial loads of these poultry feeds, which were quite high 1.232 x 10^9cfu/g (Top feeds) and 1.03 x 10^8cfu/g (Vitals feeds). Eight bacteria isolates were obtained and identified as *Bacillus* sp. [3(15.0%)], *Escherichia coli* [2(10.0%)], *Nocardia* sp. [2(10.0%)], *Salmonella* sp. [3(15.0%)], *Proteus mirabilis* [2(10.0%)], *Pseudomonas aeruginosa* [4(20.0%)], *Staphylococcus aureus* [2(10.0%)], and *Streptococcus pyogenes* [2(10.0%)]. The antibiotics susceptibility profile showed that *S. aureus* and *S. pyogenes* were more susceptible (75%) to the test antibiotics, followed by *E. coli* (72.7%), *Nocardia* sp. (58.3%) and *Proteus mirabilis* (54.5%). All gram-positive isolates were resistant to ampiclox (100%) and sensitive to streptomycin and ciprocin (100%) while all gram negative isolates were resistant to tetracycline and ampicillin (100%). However, all isolates satisfied the most common multidrug resistance patterns (≥3 antibiotics resistant). Generally, significantly higher number of multidrug-resistant *Pseudomonas* [10(90.1%)], *Bacillus* sp. [9(83.3%)], *Salmonella* sp. [8(72.7%)], *P. mirabilis* [5(45.5%)], and *Nocardia* sp. [5(41.7%)] were noted in this study. Low resistance rates was observed for *E. coli* [3(27.3%)], *S. aureus* and *S. pyogenes* [3(25%)] was found in the poultry feeds. The MIC values of tetracycline of the isolates ranged from 0.13-8.00 mcg/ml. Among all the test organisms only *S. aureus* (25%) was susceptible to tetracycline at an MIC value of 0.13 mcg/ml. This showed that 75% of the bacterial species exhibited an MIC value of 0.25-8.00 mcg/ml. To reduce the effect of these MDR organisms in poultry feeds; antibiotics incorporated into feeds should be in synergistic combinations, as this will prevent the possibility of resistance development. The findings of this study confirm the presence of multi-drug resistant organisms in animal feeds sold in Nigeria. It significantly points to the great need to evaluate and monitor the incidence rate of multi-drugs (antibiotics) resistant organisms in poultry feeds.

Key words: Antibiotics, calabar metropolis, multi-drug resistance, susceptibility profile, poultry feeds, Nigeria

INTRODUCTION

A major necessity for adequate growth (irreparable increase in body size and weight) of poultry is the provision of good and nourishing food supplements called feeds, which come in different types (e.g., starters, growers, layers, chick and finishers mash) with varying constituents (e.g. animal protein, cereals, vegetable protein, minerals, essential amino acids, salt, antibiotics, vitamin pre-mix, antioxidant, etc.) depending on the desired outcome of the poultry farmer. Poultry feeds have been presumed to have a high content of microorganisms' sequel to the manufacturing and distribution processes to adversely affect the growth and reproduction of poultry. This has therefore, necessitated the incorporation of antimicrobial agents into poultry feeds which reduces the microbial load in the field and in the gastro-intestinal tracts of the poultry, kill or inhibit infecting organisms or

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reduces the intensity of antibiotic resistance, etc. thereby improving the gross growth and quality of poultry (Ahmed, 1996).

The underlying assumption is that poultry feeds are sterile with the incorporation of antimicrobial agents. However, this incorporation poses the emergence or viability of some resistant bacteria either through genetic or non-genetic mechanisms (Gillespie, 1992). These drugs (or congeners) are also used in poultry production (e.g., each year in the United States an estimated 1.6 billion broiler eggs or chicks receive ceftiofur; *E. coli* isolates resistant to these drugs are found in poultry (Johnson *et al*., 2007). The utilization of antimicrobial drugs has played an important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion (Abdellah *et al*., 2009). The husbandry practices used in the poultry industry and the widespread use of medicated feeds in broiler and layer operations made poultry a major reservoir of antimicrobial-resistant *Salmonella* (D'Aoust *et al*., 1992). However, according to Abdellah *et al.* (2009), the extensive use of those in human and animals has led to an increase in bacterial multidrug resistant among several bacterial strains including *Salmonella*. In the developed world, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increased frequencies and dissemination of resistance genes into other ecosystems. In developing countries like Nigeria, antibiotics are used only when necessary, especially if the animals fall sick, and only the sick ones are treated in such cases (Chikwendu *et al*., 2008).

There is currently a world trend to reduce the use of antibiotics in animal food due to the contamination of meat products with antibiotic residues, as well as the concern that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria (Kabir, 2009). During the last decade, use of antimicrobial drugs for growth promotion and therapeutic treatment in food animals has received much attention. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections (Nawaz *et al*., 2009). The reservoir of resistant bacteria in food animals implies a potential risk for transfer of resistant bacteria, or resistance genes, from food animals to humans (Heuer *et al*., 2005). Subsequent emergence of infections in humans, caused by resistant bacteria originating from the animal reservoir, is of great concern. These unintended consequences of antimicrobial drug use in animals led to termination of antimicrobial growth promoters in food animals in countries in the European Union, including Denmark, where the consumption of antimicrobial drugs by production animals was reduced by 50% from 1994 to 2003 (Heuer *et al*., 2005).

However, even in the absence of heavy use of antibiotics it is important to identify and monitor susceptibility profiles of bacterial isolates, particularly of commensal organisms. This, according to John and Fishman (1997) and Chikwendu *et al.* (2008), will provide information on resistance trends including emerging antibiotic resistance, which are essential for clinical practice. This singular factor has shown the need to probe further into the prevalence of antibiotic-resistant organisms in poultry feeds sold in Nigeria. Among the antibiotics available, tetracycline has been found to be the most widely used antibiotic as an agent or constituent of poultry feeds. The presence of resistant organisms in poultry feeds indicates the potential for contamination of retail meat products. Although contamination does not necessarily mean food-borne transmission, the possibility of being a foodborne pathogen should be investigated. Hence, this study focuses on the incidence of multi-drugs resistant organisms in some poultry feeds sold in Calabar metropolis, Nigeria. It involves isolation, identification and antibiotics susceptibility profiles of microorganisms associated different poultry feeds purchased from various sales outlets in Calabar metropolis with extra emphasis on tetracycline.

MATERIALS AND METHODS

Sample collection and preparations: Duplicates samples of different poultry feeds were purchased from different sales outlets in Calabar metropolis, Nigeria. These include 50 g of Layer mash, 50 g of Finishers mash, 50 g of Starters mash, 50 g of Growers mash and 50 g of Chick mash from sale outlets of Vital feeds and Top feeds. These duplicate samples were aseptically collected in a sterile clean polyethylene bag and taken immediately to the laboratory for further bacteriological analysis as described by the methods of Fawole and Oso (2001). Ten grams (10 g) of each feeds sample was weighed out and homogenized into 90 ml of sterile distilled deionized water using a sterile warring blender. Ten fold dilutions of the homogenates were made using sterile pipettes as described by the methods of Fawole and Oso (2001).

Bacteriological analysis: All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. Media used in this study included: Nutrient Agar (NA) and Peptone Water (PW) as general and enriched media. Other media with selective and differential characteristics used were Mac Conkey Agar (MCA), Eosin Methylene Blue (EMB), Tripple Suagr Iron (TSI), Citrate Agar (CA), Christensen's Urea Agar (CUA), Mueller Hinton Agar and Mannitol Salt Agar (MSA). All media were prepared according to the manufacturer's specification and sterilized at 121ºC I bar for 15 min. From the 10-fold dilutions of the

homogenates; 0.1ml of 10^{-2}, 10^{-3} and 10^{-4} dilutions of the homogenate was plated in replicate on different media (in duplicates), using pour plate method. The plates were then incubated at 37ºC for 24-48 h. Mac Conkey agar was used for coliform enumeration while Mannitol salt agar was used for the isolation of *S. aureus*. Total viable aerobic bacteria count was performed on Nutrient Agar. At end of the incubation periods, colonies were counted using the illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (cfu/g). Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of resulting growth were then stored at 40ºC. Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests including Triple Sugar Iron (TSI) test, Indole production test, Methyl Red (MR) test, Voges-Proscauer (VP) test, Citrate utilization test, Motility Indole Urea (MIU) test, Carbohydrate fermentation test and salt tolerance test as described by Cheesbrough (2006) and Oyeleke and Manga (2008) were carried out. The isolates were identified by comparing their characteristics with those of known taxa, as described by Bergey's Manual for Determinative Bacteriology (Jolt *et al*., 1994).

Antibiotic susceptibility testing: The isolates were then subjected to antibiotic sensitivity testing by the disc diffusion method on Sheep blood agar and Mueller-Hinton agar according to the National Committee for Clinical Laboratory Standards and Manual of Antimicrobial Susceptibility Testing guidelines (NCCLS, 2004; Coyle, 2005; Cheesbrough, 2006; Okonko *et al*., 2009a, b). Commercially available antimicrobial discs were used in the study which included: ampiclox (10 µg), chloramphenicol (30 µg), erythromycin (10 µg), floxapen (30 µg), norfloxacin (30 µg), cotrimozazole (25 µg), tetracycline (25 µg), cephalexin (15 µg), ofloxacin (10 µg), reflacine (30 µg), ciprofloxacin (10 µg), penicillin (20U), gentamicin (10 µg), ampicillin (30 µg), polymixin B (30 µg), perfloxacine (10 µg), streptomycin (30 µg), lincocin (30 µg), cefuroxime (30 µg), ofloxacin (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), rifampicin (10 µg), carbencillin (30 µg), and vancomycin (10 µg). Plates were incubated at 35ºC. Zones of inhibition were interpreted as resistant or sensitive using the interpretative chart of the zone sizes of the Kirby-Bauer sensitivity test method (Cheesbrough, 2006). Multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested (Oteo *et al*., 2005). Data were analyzed using the general linear model procedure and Chi (X^2) test.

Determination of minimum inhibitory concentration (MIC) of tetracycline: A broth microdilution susceptibility assay was used, as recommended by NCCLS, for the determination of MIC (NCCLS, 2004). All tests were performed in Mueller Hinton Broth (MHB; OXOID-CM405) with the exception of the yeasts (Sabouraud Dextrose Broth-SDB; DIFCO). Bacterial strains were cultured overnight at 37ºC in Mueller Hinton Agar (MHA) and the yeasts were cultured overnight at 30ºC in Sabouraud Dextrose Agar (SDA). Test strains were suspended in MHB to give a final density of 5×10^5 cfu/ml and these were confirmed by viable counts. Geometric dilutions ranging from 1/2 mcg/ml to 1/6, 400 mcg/ml of the tetracycline were prepared in a 96 well microtiter plate, including one growth control and one sterility control. Plates were incubated under normal atmospheric conditions at 37ºC for 24 h for bacteria and at 30ºC for 48 h for the yeasts. The MIC of tetracycline was individually determined in parallel experiments in order to control the sensitivity of the test organisms. Bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

RESULTS

Table 1 shows morphological and biochemical characteristics of bacteria isolates. Twenty organisms were isolated and identified as *Bacillus* sp., *Nocardia* sp., *Escherichia coli*, *Pseudomonas aeruginosa, Proteus mirabilis*, *Salmonella* sp., *Staphylococcus aureus* and *Streptococcus pyogenes*.

Table 2 shows the frequency of occurrence of organisms isolated from poultry feeds sold in Calabar metropolis, Nigeria. It showed that *Pseudomonas aeruginosa* [4 (20.0%)] was most predominant. This was closely followed by *Bacillus* sp. [3(15.0%)], *Salmonella* sp. [3(15.0%)], *Nocardia* sp. [2(10.0%)], *Escherichia coli* [2(10.0%)], *Proteus mirabilis* [2(10.0%)], *Staphylococcus aureus* [2(10.0%)] and *Streptococcus pyogenes* [2(10.0%)].

Table 3 shows the microbial loads of poultry feeds sold in Calabar metropolis, Nigeria. The mean microbial load of the feeds from location 1 (Top feeds) ranged between $0.65x10^7$-$1.232x10^9$cfu/g while location (Vitals feeds) ranged between $0.95x10^7$-$1.03x\ 10^8$cfu/g as shown in Table 3. From location 1 (Vitals), samples of layers mash had a mean total viable count of $1.232x10^8$ cfu/g, finishers, growers, and chick mash had $0.95x10^7$ cfu/g, $0.65x10^7$cfu/g and $0.99x10^8$cfu/g respectively. However, layer mash had significantly ($p<0.05$) higher viable count ($1.232x10^9$ cfu/g) compared to other feeds from this location (Table 3). Among the feed samples collected from location 1 (Top), growers mash had the highest total viable count ($1.03x10^8$ cfu/g), starter mash ($0.95x10^7$ cfu/g), finishers and layers mash had $3.25x10^7$ and $0.37x10^8$ cfu/g, respectively. However, grower mash

Table 1: Morphological and biochemical characteristics of bacteria isolates

	Isolates							
Parameters	BS	NS	SA	PA	EC	SS	SP	PM
Grams reaction	+	+	+	-	-	-	+	-
Cellular morphology	Rods	Branching rods	Cocci	Small rods	Straight rods	Rods	Cocci in chains	Small rods
Growth on blood agar (colony)	N/A	Large grayish-white partially mucoid	Creamy white	Large flat, spreading & a dark greenish	Large, flat spreading & circular mucoid	Greyish-white mucoid	Creamy/colourless, mucoid in chains with zones of complete haemolysis	Swarming with characteristic fishy smell
Growth on MacConkey agar	Pink	Mucoid	N/A	Pale	Smooth Red/Pink	Pale	Pink	Colourless
Growth on Mannitol salt agar	N/A	N/A	Bright yellow	N/A	N/A	N/A	N/A	N/A
Growth on Sabouraud agar	N/A	Pinkish wavy folded -like, whitish wire-like structure	N/A	N/A	N/A	N/A	N/A	N/A
Motility	-	-	-	+	-	+	-	+
Catalase test	+	+	+	+	+	+	-	+
Coagulase test	N/A	N/A	+	N/A	N/A	N/A	-	N/A
Citrate test	+	-	+	+	+	+	+	+
Oxidase test	N/A	-	-	+	-	-	-	-
Indole test	-	-	-	-	+	-	-	-
Urease activity	N/A	+	+	-	-	-	-	+
Methyl Red	+	+	+	-	-	+	+	+
Voges Proskauer	+	-	-	+	-	-	-	-
Bacitracine	N/A	N/A	N/A	N/A	N/A	N/A	+	N/A
Growth on TSI Medium								
Slope	N/A	N/A	Yellow	Yellow	Yellow	Yellow	Yellow	Red-pink
Butt	N/A	N/A	Yellow	A	Yellow	Yellow	Yellow	Yellow
Hydrogen Sulphide (H_2S)	N/A	N/A	+	-	-	+	+	+
Gas production	N/A	N/A	-/G	-	-/G	-/G	-/G	-/G
Sugar fermentation test								
Glucose	A/-	A/G	A/G	-/-	A/G	A/G	A/G	A/G
Lactose	A/-	-/-	A/-	A/G	A/-	A/G	-	-/-
Sucrose	A/-	-/-	A/-	A/G	A/-	A/G	A/G	A/-
Mannitol	A/-	-/-	A/-	A/G	A/-	A	-/-	-/-
Maltose	A/-	-/-	A/-	-/-	A/-	A/G	-/-	-/-
Most probable organism	*Bacillus* sp.	*Nocardia* sp.	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*E. coli*	*Salmonella* sp.	*Streptococcus pyogenes*	*Proteus mirabilis*

Keys:
N/A = Not applicable, - = No growth, + = Growth, A/G = Substrate utilization with acid and gas production, A/- = Substrate utilization with acid production only and no gas production, -/G = Substrate utilization with gas production only, -/- = No substrate utilization, Yellow = Acidic reaction, Red-pink = Alkaline reaction

Table 2: Frequency of occurrence of isolates

Isolates	No. (%)
Bacillus sp.	3 (15.0)
Nocardia sp.	2 (10.0)
Staphylococcus auerus	2 (10.0)
Streptococcus pyogenes	2 (10.0)
Escherichia coli	2 (10.0)
Proteus mirabilis	2 (10.0)
Pseudomonas aeruginosa	4 (20.0)
Salmonella sp.	3 (15.0)
Total	20 (100.0)

had significantly ($p<0.05$) higher viable count ($1.03x10^8$cfu/g) compared to other feeds from this location (Table 3). Also, there were significant difference ($p<0.05$) between the mean microbial loads of the two locations.

Table 4a, b and c show the antibiotics susceptibility profiles of the isolates. The isolates exhibited a wide range of resistance and sensitivity to respective gram positive (Table 4a) and gram-negative (Table 4b) antibiotics and overall comparison (Table 4c). Multidrug resistance (MDR) was defined as resistance to ≥3 of the antimicrobial agents tested (Oteo *et al.*, 2005). From 4a, MDR-*Bacillus* sp. (83.3%) was observed as it showed resistant to all test antibiotics except Streptomycin and Ciprocin (resistant to 10 of 12 antibiotics), thereby showing 16.7% sensitivity (sensitive to only 2 antibitotics). *Nocardia* sp.. was resistant to 5 antibiotics (41.7%) and sensitive 7 antibiotics (58.3%). Lower resistant rates (25%) were observed in *S. aureus* and *S. pyogenes*. Both were resistant to 3 antibiotics and sensitive to 9 (75%) antibiotics. *S. aureus* was resistant to ampiclox, penicillin, and rifampicin all test antibiotics except for, and while *S. pyogenes* was resistant to ampiclox, erythromycin, and tetracycline (Table 4a). Only *S. pyogenes* was susceptible to penicillin.

Table 4b shows the antibiotic susceptibility profiles of gram-negative isolates. Percentages of resistance observed among the gram negative isolates were 27.3%, 45.5%, 72.7%, and 90.1% (*E. coli*, *P. mirabilis*, *Salmonella* sp. and *P. aeruginosa* respectively). All showed MDR pattern and all except *E. coli* were resistant to chloramphenicol and carbencillin (75%). *P. aeruginosa* was resistant (90.1%) to all test antibiotics except gentamicin (9.1%). *Salmonella* sp. was resistant to 8 antibiotics; virtually all test antibiotics except 3 antibiotics (27.3%), which include contrimoxazole, ciprofloxacin and reflacine (Table 4b). Generally, all gram-positive isolates were resistant to ampiclox (100%) and sensitive to streptomycin and ciprocin (100%) as shown in Table 4a.

Table 4c shows the overall frequency of antibiotic susceptibility profiles of all isolates. High percentage of

Table 3: Microbial loads of poultry feeds sold in calabar metropolis, Nigeria

Location	Sample	Mean total viable count (Cfu/g)
Vital feeds	Layer mash (LM)	0.37×10^8
	Finisher mash (FM)	3.25×10^7
	Starter mash (SM)	0.95×10^7
	Grower mash (GM)	1.03×10^8
Top feeds	Layer mash (LM)	1.232×10^9
	Finisher mash (FM)	0.95×10^7
	Chick mash (CM)	0.99×10^8
	Grower mash (GM)	0.65×10^7

Table 4a: Antibiotic susceptibility profiles of gram-positive isolates

	Gram positive isolates			
Antibiotics (µg)	*Bacillus* sp..	*Nocardia* sp..	*S. auerus*	*S. pyogenes*
Rifampicin (10)	R	S	R	S
Erythromycin (10)	R	S	S	R
Tetracycline (25)	R	R	S	R
Chloramphenicol (25)	R	R	S	S
Ampiclox (10)	R	R	R	R
Streptomycin (30)	S	S	S	S
Penicillin (20U)	R	R	R	S
Floxapen (30)	R	S	S	S
Norfloxacin (30)	R	S	S	S
Lincocin (30)	R	S	S	S
Ciprocin (10)	S	S	S	S
Vancomycin (10)	R	R	S	S
No. of sensitive (%)	2 (16.7)	7 (58.3)	9 (75.0)	9 (75.0)
No. of resistant (%)	10 (83.3)	5 (41.7)	3 (25.0)	3 (25.0)

S = Sensitive; R = Resistant

Table 4b: Antibiotic susceptibility profiles of gram negative isolates

	Gram-negative isolates			
Antibiotics (µg)	*E. coli*	*P. mirabilis*	*P. aeruginosa*	*Salmonella* sp.
Tetracycline (25)	R	R	R	R
Ampicillin (25)	R	R	R	R
Gentamicin (10)	R	S	S	R
Contrimoxazole (25)	S	S	R	S
Ofloxacin (10)	S	S	R	R
Ciprofloxacin (30)	S	S	R	S
Ceflaximide (10)	S	S	R	R
Polymixin B (30)	S	S	R	R
Carbencillin (30)	S	R	R	R
Reflacine (30)	S	R	R	S
Chloramphenicol (25)	S	R	R	R
No. Sensitive (%)	8 (72.7)	6 (54.5)	1 (09.1)	3 (27.3)
No. Resistant (%)	3 (27.3)	5 (45.5)	10 (90.9)	8 (72.7)

S = Sensitive; R = Resistant

resistance was found to the following antimicrobial agents: ampicillin (100%), ampiclox (100%), tetracycline (87.5%), carbencillin (75%), penicillin (75%), chloramphenicol (62.5%), ceflaximide, erythromycin, gentamicin, ofloxacin, polymixin, reflacine, rifampicin and vancomycin (50%). Low resistance rates were recorded for ciprofloxacin, contrimoxazole, floxapen, lincocin and norfloxacin (25%), while zero resistance were recorded against streptomycin. On the other hand 100% of isolates were found to be sensitive to streptomycin and 75% to ciprofloxacin, contrimoxazole, floxapen, lincocin, norfloxacin, however 50% sensitivity was observed for ceflaximide, erythromycin, gentamicin, ofloxacin, polymixin, reflacine, rifampicin, and vancomycin. Low sensitivities were recorded against chloramphenicol (37.5%), carbencillin and penicillin (25%), and tetracycline (12.5%), while zero sensitivity was recorded against ampicillin and ampiclox.

The degree of growth of each bacterial species with respect to sensitivity or resistance to tetracycline at different concentrations is shown in Table 5. Isolates from vitals- and top- feeds plates underwent broth dilution MIC determinations with tetracycline regardless of disk test results. On disk diffusion susceptibility testing, the isolates were resistant to tetracycline except for *S. aureus* with MIC value of 0.13 mcg/ml (25%). The MIC of tetracycline was as high as 8.00 mcg/ml. Generally, the MIC values of tetracycline ranged from 0.13-8.00 mcg/ml

Table 4c: Overall frequency of antibiotic susceptibility profiles of all isolates

Antibiotics (µg)	No. of isolates tested	No. of sensitive (%)	No. of resistant (%)
Ampicillin (25)	4	0 (00.0)	4(100.0)
Ampiclox (10)	4	0 (00.0)	4(100.0)
Carbencillin (30)	4	1(25.0)	3(75.0)
Ceflaximide (10)	4	2 (50.0)	2 (50.0)
Chloramphenicol (25)	8	3 (37.5)	5 (62.5)
Ciprocin (10)	4	4(100.0)	0 (00.0)
Ciprofloxacin (30)	4	3(75.0)	1(25.0)
Contrimoxazole (25)	4	3(75.0)	1(25.0)
Erythromycin (10)	4	2 (50.0)	2 (50.0)
Floxapen (30)	4	3(75.0)	1(25.0)
Gentamicin (10)	4	2 (50.0)	2 (50.0)
Lincocin (30)	4	3(75.0)	1(25.0)
Norfloxacin (30)	4	3(75.0)	1(25.0)
Ofloxacin (10)	4	2 (50.0)	2 (50.0)
Penicillin (20U)	4	1(25.0)	3(75.0)
Polymixin B (30)	4	2 (50.0)	2 (50.0)
Reflacine (30)	4	2 (50.0)	2 (50.0)
Rifampicin (10)	4	2 (50.0)	2 (50.0)
Streptomycin (30)	4	4(100.0)	0 (00.0)
Tetracycline (25)	8	1(12.5)	7(87.5)
Vancomycin (10)	4	2 (50.0)	2 (50.0)

Table 5: Minimum inhibitory concentration of tetracycline for poultry feeds isolates

S. No.	Code Vital feeds	Isolates	MIC value (mcg/ml)
1	Layers Mash 1	*Bacillus* sp.	4.00
2	Layers Mash 2	*Salmonella* sp.	2.00
3	Layers Mash 3	*Staphylococcus aureus*	0.13
4	Finishers Mash 1	*Escherichia coli*	0.25
5	Finishers Mash 2	*Streptococcus pyogenes*	0.25
6	Starters Mash 1	*Pseudomonas aeruginosa*	4.00
7	Starters Mash 2	*Nocardia* sp.	1.00
8	Growers Mash 1	*Pseudomonas aeruginosa*	4.00
9	Growers Mash 2	*Bacillus* sp.	4.00
	Top feeds		
1	Layers Mash 1	*Salmonella* sp.	4.00
2	Layers Mash 2	*Streptococcus pyogenes*	0.50
3	Layers Mash 3	*Pseudomonas aeruginosa*	4.00
4	Layers Mash 4	*Bacillus* sp.	8.00
5	Finishers Mash 1	*Salmonella* sp.	4.00
6	Finishers Mash 2	*Proteus mirabilis*	4.00
7	Growers Mash 1	*Proteus mirabilis*	1.00
8	Growers Mash 2	*Nocardia* sp.	4.00
9	Chicks Mash 1	*Staphylococcus aureus*	0.13
10	Chicks Mash 2	*Escherichia coli*	0.25
11	Chicks Mash 3	*Pseudomonas aeruginosa*	4.00

and 75% of the isolates exhibited an MIC value of 0.25-8.00 mcg/ml (Table 5).

DISCUSSION

The widespread use of antibiotics in animals has also raised several concerns related to human and animal health. The principal area of concern has been the increasing emergence of antibiotic resistance phenotypes in both clinically relevant strains and normal commensal microbiota (Chikwendu *et al.*, 2008). The microbial isolates characterized and identified include eight bacterial genera, among which are the bacterial isolates: *P. aeruginosa*, *Bacillus* sp.., *Salmonella* sp.., *Nocardia* sp., *E. coli*, *P. mirabilis*, *S. aureus* and *St. pyogenes*. The degree of frequency of microbial distribution was high in the top feeds. Oyeleke (2009) also isolated similar organisms (*Staphylococcus*, *Enterobacter* and *Bacillus*) in his study on yoghurt commercially produce in Minna. *Staphylococcus* is known to be easily carried in the nasopharynx, throat, skin, cuts, boils, nails and as such can easily contribute to the normal microflora (Ekhaise *et al.*, 2008). Mordi and Momoh (2009) reported 17.3% incidence of *Proteus mirabilis* in their study on wound infection in Benin City, Nigeria, which is comparable to our present finding. *P. mirabilis* isolated in this study is less than 90%, as claimed by Auwaerter (2008).

From this study, *P. aeruginosa*, *Bacillus* sp., and *Salmonella* sp. appeared to be the most prevalent bacteria species. It is therefore important to evaluate the quality of the feeds we give to poultry animals. The number and

type of food-borne microorganisms can be used to determine the degree of quality, cleanliness, purity as well as to determine the source of contamination and infection. Higher prevalence of commensal flora is known to contribute to the general increase and dissemination of bacterial resistance worldwide and can be a source of resistance genes for respiratory pathogens such as *Streptococcus pneumoniae* and intestinal pathogens like *Shigella* and *Salmonella* (Chikwendu *et al*., 2008).

The microbial load of $1.232x10^9$ and $1.03x10^8$ cfu/g found in these poultry feeds were quite on the high side. Similar total bacterial count ranging from $1.0x10^7$ to $9.4x10^7$cfu/ml was also reported in a study on commercially produced yoghurt by Oyeleke (2009). The higher microbiological counts in the top feeds samples compared to the vitals feeds samples possibly demonstrates a wide variation of poor hygiene practices in the productions. The level of contamination of the poultry feeds sample is enormous and indicator of recent faecal contamination and gross pollution of the feeds production plants. *E. coli*, which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *E. coli* and the *E. coli* 0157: H7 strain has previously been isolated from meat samples (Oyeleke, 2009). The presence of these organisms in these poultry feeds depicts a deplorable state of poor hygienic and sanitary practices employed in the manufacturing, processing and packaging of animal feeds.

The findings of this study has shown that there was faecal contamination of poultry feeds both vitals and top sources as illustrated by the presence of the test organisms. The high incidence of *E. coli* (10%) in poultry feeds is a concern as such sources are usually regarded as "safe". The presence of these 2 organisms *E. coli* (10%) and *Salmonella* sp. (15%) demonstrates a potential health risk as the organism is pathogenic and causes complications in humans and animals. *E. coli* and *Salmonella* contamination is usually associated with contaminated water and food (animal feeds) and their presence reflects the degree of purity or signals faecal contamination of both human and animal origin (Ekhaise *et al*., 2008). *E. coli* is coliforms which make-up approximately 10% of microorganism of the humans and is used as the indicator organisms of faecal contamination and is associated with poor environmental sanitation (Prescott *et al*., 2005). However, many drug-resistant human fecal *E. coli* isolates may originate from poultry, whereas drug-resistant poultry-source *E. coli* isolates likely originate from susceptible poultry-source precursors (Johnson *et al*., 2007).

In this study, all gram-positive isolates were resistant to ampiclox (100%) and sensitive to streptomycin and ciprocin (100%) while all gram-negative isolates were resistant to tetracycline and ampicillin (100%). MDR-*Pseudomonas* (90.1%) has been previously reported. The ability of MDR-*Bacillus* sp. (83.3% resistance) to form spores could probably explain its resistant potential to antibiotics and tolerate very low moisture conditions found in poultry feeds. *Nocardia* sp. with 41.7% resistance (5 antibiotics), was also on the high side. Low percentage resistance, 25% observed for *S. aureus* and *S. pyogenes* is quite a good one, though both cases satisfied multidrug resistant pattern (resistant to 3 antibiotics). The proportion of isolates with *in vitro* resistance to erythromycin has increased since 1996 (Motlová *et al*., 2004). In this study, *S. aureus* was resistant to penicillin, ampiclox and rifampicin. Ineffectiveness of penicillin and ampicillin against *S. aureus* has been reported by Suchitra and Lakshmidevi (2009) and Okonko *et al*. (2009a). Also, Okonko *et al*. (2009a) in their study reported ineffectiveness of tetracycline and ampicillin (ampiclox) against *S. pyogenes*. This agrees with our present findings. According to Suchitra and Lakshmidevi (2009), intensive medical therapies and frequent use of antimicrobial drugs are capable of selection of resistant microbial flora. This also points to the fact that the prevalence of such multidrug resistant organisms should be checkmated since their economic implication cannot be over emphasized.

Among the gram-negative isolates, the *P. mirabilis* isolated in this study were sensitive to ofloxacin and ciprofloxacin of the fluorinated quinolone group and gentamycin an aminoglycoside. This is in agreement with what was reported by Mordi and Momoh, (2009). These antibiotics are therefore recommended as an effective single broad-spectrum antibiotic both in empirical and prophylactic treatment. Also in this study, 45.5% resistant to 5 (ampicillin, chloramphenicol, carbencillin, reflacine and tetracycline) out of 11 antibiotics *in vitro* was observed for *P. mirabilis* in this study, but 55.5% sensitive to 6 others. MDR *P. mirabilis* with 45.5% resistance (5 antibiotics) was also on the high side. Sensitivity of *P. mirabilis* to ciprofloxacin, ofloxacin, and gentamicin, and its resistance to tetracycline reported by Mordi and Momoh (2009) is similar to our present finding. According to Mordi and Momoh (2009), literature reports indicated that most strains of *Proteus* are susceptible to cotrimoxazole and almost all species are sensitive to gentamicin. However, the *in vitro* sensitivity in this study did show gentamicin and cotrimoxazole to be the drug of choice for *Proteus* infections as well as the quinolones, ciprofloxacin and oflxacin.

Proteus species show a characteristic swarming motility, which is observed, on non-inhibitory agar medium as a wave-like movement across the entire surface of agar medium. Whenever swarming is observed *Proteus* species should be suspected. *P. mirabilis* is the species most commonly recovered from humans, especially from urinary and wound infections. It accounts

for 90% of all infections caused by the *Proteus* species (Auwaerter, 2008). It is however not involved in nosocomial infections as do the indole positive species (Auwaerter, 2008). Though, in the literature, sensitivity to chloramphenicol was a way of differentiating *P. mirabilis* from *P. vulgaris* in being sensitive to chloramphenicol and ampicillin. According to Gus-Gunzalez *et al.* (2006 reviewed in Mordi and Momoh, 2009), *P. mirabilis* is differentiated from other species by been indole negative, chloramphenicol and ampicillin sensitive and ability to produce hydrogen sulphide. This is contrary to our present finding. In this study, it was observed that all the *P. mirabilis* were resistant to both ampicillin and chloramphenicol. This observation can be explained on the widespread plasmid resistance genes among *Proteus* species (Yah *et al.*, 2007; Enabulele *et al.*, 2009; Auwaerter, 2008; Mordi and Momoh, 2009). The possibility of observing more than one gram negative isolate in a clinical, environmental or food sample facilitates the exchange of plasmid resistance genes between organisms.

Also, the *P. mirabilis* resistance to ampicillin, chloramphenicol, carbencillin, reflacine and tetracycline could be a result of the extra outer cytoplasmic membrane which contains a lipid bilayer, lipoproteins, polysaccharides and lipopolysaccharides, and of course, abuse and misuse of antibiotics could be part of the contributing factors of resistance to antibiotics. It is advisable that treatment of *Proteus* infection be guided by the sensitivity result since the antibiotic susceptibility pattern of each species, depends on the extent to which the use of the various drugs has either selected resistant mutant or promoted the transfer of resistance factor from other members of the enterobacteriaceae (Yah *et al.*, 2007; Mordi and Momoh, 2009).

As members of the enterobacteriaceae, they are all oxidase negative, actively motile, non spore forming, non capsulated and are recognized by their ability to cause disease (Auwaerter, 2008). They cause significant clinical infections, which are difficult to eradicate especially from hosts with complicated wounds, catheterization and underlying diseases and the immuno-compromised (Auwaerter 2008; Mordi and Momoh, 2009). However, *Proteus* species, which colonize the intestinal tract, differ from those found in wounds in their ability to carry genes encoding antibiotic resistance (Yah *et al.*, 2007). The routine use of antibiotics in both medical and veterinary medicine has resulted in wide spread antibiotic resistance and development of antibiotic resistance genes especially within the gram-negative organisms (Mordi and Momoh, 2009). As members of the genus *Proteus* occur widely in man, animals and in the environment and can be readily recovered from sewage, soil, garden vegetables and many other materials, it is necessary to control the organisms since the sources of infection are many (Mordi and Momoh, 2009).

Today *Proteus* organisms are known to cause significant clinical infections and occupy multiple environmental habitats (Mordi and Momoh, 2009). The varying pattern of microbial isolates among different studies emphasized the need for surveillance to evaluate and monitor periodically the changing pattern of the microflora especially in a hospital setting (Mordi and Momoh, 2009). As *Proteus* species are found in multiple environmental habitats including long term care facilities and hospitals, and they cause significant clinical infections, it becomes necessary to have a continued surveillance of the organisms. Knowledge of their presence in an environment and their sensitivity pattern are important tools in the management of infections in animals and in humans (especially wound infections in humans), and are also useful in formulating rational antibiotic policy (Mordi and Momoh, 2009).

Also, in this study, low percentage resistance rate of 27.3% was observed for *E. coli*. This is also quite a good one, though it satisfied multidrug resistant pattern of resistance to $\geq$3 antibiotics (ampicillin, gentamicin, and tetracycline). The MDR pattern reported on *E. coli* in this study is comparable to previous studies (Dolejska *et al.*, 2007; Sjölund *et al.*, 2008). However, gentamicin resistant *E. coli* observed in our study is contrary to the zero gentamicin resistance reported by Sjölund *et al.* (2008). Ineffectiveness of penicillin G and ampicillin against *E. coli* has also been reported (Dolejska *et al.*, 2007; Sjölund *et al.*, 2008; Okonko *et al.*, 2009a). Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (Karlowsky *et al.*, 2004). In recent years, fluoroquinolone resistance has increased in some countries and reports of multidrug resistance are not infrequent (Karlowsky *et al.*, 2004; Sherley *et al.*, 2004). It is estimated that nearly 90% of all antibiotic agents use is in food animals, are given at subtherapeutic concentrations prophylactically or to promote growth (Abdellah *et al.*, 2009). Antimicrobial drug resistance in *E. coli* isolated from wild birds has been described by Dolejska *et al.* (2007).

Our findings on *E. coli* showed no close resemblance to those of a recent study of ciprofloxacin-resistant *E. coli* from humans and chickens in the late 1990s in Barcelona, Spain reported by Johnson *et al.* (2007) as no ciprofloxacin-resistant *E. coli* was reported in this study. However, acquired resistance to first-line antimicrobial agents increasingly complicates the management of extra-intestinal infections due to *E. coli*, which are a major source of illness, death, and increased healthcare costs (Johnson *et al.*, 2007). One suspected source of drug-resistant *E. coli* in humans is use of antimicrobial drugs in agriculture (Johnson *et al.*, 2007). This use presumably selects for drug-resistant *E. coli*, which may be transmitted to humans through the food supply (Johnson *et al.*, 2007). Supporting this hypothesis is the

high prevalence of antimicrobial drug-resistant *E. coli* in retail meat products, especially poultry reported by Schroeder *et al.* (2005) and Johnson *et al.* (2007), and the similar molecular characteristics of fluoroquinolone-resistant *E. coli* from chicken carcasses and from colonized and infected persons in Barcelona, Spain, in contrast to the marked differences between drug-susceptible and drug-resistant source isolates from humans (Johnson *et al*., 2007). Such multidrug resistance has important implications for the empiric therapy of infections caused by *E. coli* and for the possible co-selection of antimicrobial resistance mediated by multidrug resistance plasmids (Sherley *et al*., 2004).

In this present study, *P. aeruginosa* was resistant to 10 (90.1%) antibiotics and sensitive to gentamicin only. The presence of MDR *Pseudomonas aeruginosa* in these poultry feeds is another cause for concern since it is commonly found in chronic lung infections such as cystic fibrosis and others. Intrinsic antibiotic resistance of *P. aeruginosa* accompanied by its ability to acquire resistance via mutations and adapt to the heterogeneous and dynamic environment are major threats and reasons for the ultimate failure of the current antibiotic therapies in eradicating the infection from lungs. It is virtually impossible to completely eradicate a chronic *P. aeruginosa* respiratory infection, which is frequently observed to accompany respiratory diseases such as cystic fibrosis, bronchiectasis and Chronic Obstructive Pulmonary Disease (COPD) (Seshadri and Chhatbar, 2009). Ability of *P. aeruginosa* to escape the effects of antibiotics is attributed by its capability of growing in microaerophilic environment as biofilms both of which reduce the efficiency of many antibiotics (Seshadri and Chhatbar, 2009). Major concern however, in case of *P. aeruginosa* is combination of its inherent resistance and ability to acquire resistance via mutations to all treatments leading to increasing occurrence of multi drug resistant strains (Henrichfreise *et al*., 2007).

In this study, *P. aeruginosa* was resistant to all test antibiotics except gentamicin. Okonko *et al.* (2009a) reported a similar MDR trend as observed in this present study. Some studies have shown *P. aeruginosa* was 100% resistant to gentamicin (Seshadri and Chhatbar, 2009) and 100% sensitive to ofloxacin and ciprofloxacin (Okonko *et al*., 2009a), which were some of the antibiotics used for antimicrobial prophylaxis. Okonko *et al*. (2009a) reported that *P. aeruginosa* was 66.7% resistant to gentamicin. These were contrary to our present finding which showed that *P. aeruginosa* was 100% sensitive to gentamicin, though our study agrees with their 100% resistance to ampicillin, chloramphenicol, cotrimoxazole, and tetracycline. In the present study, the percentage resistance of MDR *P. aeruginosa* strains from poultry feeds was 90.1%. However, our worst nightmare may even be more dreadful, with the occurrence of "panresistant" strains that arise due to the accumulation of multiple mechanisms of antibiotic resistance (Seshadri and Chhatbar, 2009).

Lastly, among the gram-negative isolates, *Salmonella* spp. are among the most common causes of human bacterial gastroenteritis worldwide, and food animals are important reservoirs of the bacteria (Skov *et al*., 2007). It is recognized worldwide as important pathogens in the intestinal tracts of both animals and humans. Poultry has also been considered an important source of *Salmonella* Mbandaka for humans (Filioussis *et al*., 2008). However, *Salmonella* infections are the most common cause of bacterial diarrhea in humans (Gallay *et al*., 2007) and animals worldwide. Locally compounded poultry feeds have been implicated in the spread of MDR *Salmonella* sp. among poultry and possible transmission to humans. In recent years, an increase in the occurrence of antimicrobial drug-resistant *Salmonella* spp. has been observed in several countries (Skov *et al*., 2007). Mbuko *et al*. (2009) in a study conducted in Zaria Nigeria, reported 18.4% fowl typhoid (FT) cases (an infection caused by *Salmonella enterica* serovars Gallinarium (Abdu, 2007), usually following the ingestion of food (feeds) or water contaminated by the fecal material) in among chickens (98.4%) and 9.4% in Jos (Okwori *et al*., 2007). All the authors attributed these prevalence rates to increase in the number of backyard poultry farmers that are compounding feeding by themselves (Abdu, 2007; Mbuko *et al*., 2009). The clinically infected birds are carriers of FT, which can also be transmitted by attendants through hands, feet, clothes and rodents or via eggs laid by vaccinated birds (Abdu, 2007; Okwori *et al*., 2007; Mbuko *et al*., 2009). So care should be taking to ensure that feeds used in poultry are of standard quality (microbiologically).

In this study, *Salmonella* sp. was resistant to 8 (72.7%) out of the 11 antibiotics *in vitro* (ampicillin, chloramphenicol, gentamicin, ofloxacin, ceflaximide, polymixin B, carbencillin, and tetracycline), and 27.3% sensitive to 3 antibiotics (cotrimoxazole, ciprofloxacin and reflacine). The antimicrobial susceptibility patterns of the *Salmonella* strains isolated indicated that a large proportion of the isolates were resistant to a variety of the drugs tested particularly tetracycline. The percentages of resistance obtained with these antibiotics are comparable with those reported in other studies in France, Senegal and Morocco (Abdellah *et al*., 2009). Ineffectiveness of ampicillin, chloramphenicol, gentamicin, ofloxacin, ceflaximide, polymixin B, carbencillin, and tetracycline against *Salmonella* sp. has been previously reported (Adachi *et al*., 2005; Oteo *et al*., 2005; Filioussis *et al*., 2008).

MDR *S. typhimurium* strains have been well documented in food animals, as have MDR *S. Typhimurium* outbreaks in humans from animal contact or

foods of animal origin (Wright *et al*., 2005). Abdellah *et al*. (2009) reported 75.43% resistivity by *Salmonella* isolates to one or more antimicrobials and 39.5% multiple resistances to two or more different antimicrobials. Resistance to tetracycline was the most frequent in their study. Their study found *Salmonella* isolates to be resistant to several antimicrobials. It should be noted that the presence and distribution of resistant *Salmonella* serotypes could vary from region to region and that isolation rates depend upon the country where the study was carried out, the sampling plan and the detection limit of the methodology (Abdellah *et al*., 2009).

A prominent reason for concern with regard to these MDR organisms is the recognized emergence of antimicrobial resistance among key species. Over the past decade, particularly in developing countries, the increase in resistance of animal origin nontyphoid salmonellae to broad-spectrum antibiotics such as cephalosporins, tetracycline, and quinolones has been extremely worrisome (Filioussis *et al*., 2008). As a result, attempts are being made to trace salmonellosis outbreaks to contaminated sources, and numerous typing methodologies have been used (Filioussis *et al*., 2008). However, a number of studies in the literature indicated a gradual increase in the emergence of antibiotic-resistant microorganisms especially in hospitals (Suchitra and Lakshmidevi, 2009). Many factors apart from antibiotic exposure can contribute to the development of antibiotic resistance in bacterial isolates. Major among these are environmental stress, heat stress in swine, increased propulsion of intestinal content causing a reduction in transit time, which, in turn, increases shedding of resistant *E. coli*. Overcrowding in holding pens also increases the percentage of antimicrobial resistant enteric bacteria shed into the environment by pigs. Other factors that may disturb gastrointestinal microflora are starvation or other dietary changes, fear and other conditions like cold (Chikwendu *et al*., 2008).

The results outlined above significantly point out to the fact that there is a great need to evaluate and monitor the incidence rate of multi-drugs (antibiotics) resistant organisms in poultry feeds. Since antibiotics were usually incorporated into poultry feeds, it would be logically correct to assume that every bacterial cell isolated from poultry feeds has the genes for emerging drug resistance. However, the susceptibility profiles revealed a high resistance pattern to the antibiotics used. The isolates exhibited a wide range of resistance and sensitivity to respective gram- positive and -negative antibiotics. Most organisms isolated in this study showed varying degree of resistance ranging from 3 to 10 antibiotics. Multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested (Oteo *et al*., 2005), which revealed the multi-drug resistant pattern and ability of these organisms to many antimicrobials (3 to 10 antibiotics) with resistance rates changing from 25% to 90.1%. Surveillance of Campylobacter antimicrobial drug resistance was implemented in France in 1999 for broilers in conventional and free-range broiler farms and in 2000 for pigs as part of a surveillance program on resistance in sentinel bacteria (*Escherichia coli* and *Enterococcus* spp.) and zoonotic bacteria (*Salmonella* spp. and *Campylobacter* spp.) in animal products for human consumption (Gallay *et al*., 2007).

In the overall, the most common multidrug resistance (≥3 drugs) patterns included resistance to penicillin, carbencillin, ampicillin or ampiclox, chloramphenicol and tetracycline. Resistance to ciprofloxacin, contrimoxazole, floxapen, lincocin, norfloxacin, remained low, and no isolate was resistant to ciprocin and streptomycin. Resistance to ampicillin is of clinical interest because this drug may be used for the treatment of severe infections. Cotrimoxazole resistance in line with other studies in recent time remained stable (25%), Oteo *et al*. (2005) reported approximately 30% and 100% was reported by Okonko *et al*. (2009a) in clinical isolates in Abeokuta, Nigeria. Possibly the most important determining factor in resistance is use of antimicrobial agents, as described for ciprofloxacin (Bolon *et al*., 2004).

The Minimum Inhibitory Concentration (MIC) values of tetracycline of the isolates ranged from 0.13-8.00 mcg/ml. The isolates were resistant to tetracycline except for *S. aureus* with MIC value of 0.13 mcg/ml (25%). The MIC of tetracycline was as high as 8.00 mcg/ml. One remarkable observation is that a predominant percentage (75%) of the isolates had an MIC value above 0.13 mcg/ml concentration. The latter threshold corresponds with susceptibility shown with disk test and includes isolates with potentially clinically relevant reduced susceptibility. A critical look at the MIC of tetracycline for these MDR organisms revealed that probably the concentration of the antibiotics incorporated were not significant enough to have bacteriocidal or bacteriostatic effects on the cells. *Pseudomonas aeruginosa* with MIC value of 4 mcg/ml has the ability to resist many antimicrobials while *Bacillus* sp. with MIC value of 8 mcg/ml form spores that are probably resistant to the antibiotic (tetracycline) and can tolerate very low moisture conditions as found in these poultry feeds. Other bacteria that are not spore formers found in these poultry feeds with MIC value ranging from 0.25-4.00 mcg/ml may have produced plasmid-coded beta-lactamase or evolved certain resistance mechanisms to curb the effect of tetracycline and they can probably exist in dehydrated forms inside the feed and remain subjected to regeneration with respect to metabolic activities upon introduction to moisture in the gastro-intestinal tract of the poultry.

Generally, a significantly higher number of resistant and multidrug-resistant *Salmonella*, *Bacillus*, and

Pseudomonas isolates were found among isolates from poultry feeds. Multi-drugs resistant organism has been frequently reported even when concentration of the incorporated antibiotics were high enough, in theoretical terms, to inhibit microorganisms. This indicates that probably the potencies of the antibiotics purchased and incorporated may be too low or the rigorous processes involved in manufacturing feeds has relatively reduced the effectiveness of the antibiotics. There is therefore an urgent need to evaluate and ensure the use of antibiotic (s) in synergistic combinations to produce good results and reduce the possibility of development or emerging resistance. Combined studies in humans and poultry have implicated the use of fluoroquinolones in poultry in the emergence of drug resistance. As a consequence, in 2004 the US Food and Drug Administration withdrew the 1995/1996 approvals for the new animal drug application to use enrofloxacin for prophylaxis treatment or growth promotion in poultry. Veterinary licensing of enrofloxacin in poultry was approved by the European Union (EU) in 1991, and in 1999 the EU recommended limiting the use of fluoroquinolones in poultry (Gallay *et al*., 2007).

The presence of multidrug resistant pathogenic organisms such as *Bacillus* sp., *Nocardia* sp., *E. coli*, *P. mirabilis*, *P. aeruginosa*, *Salmonella* sp., *S. aureus* and *S. pyogenes* encountered in these poultry feeds is alarming. The presence of these organisms in these poultry feeds should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication (Kabir, 2009). The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents (Alim *et al*., 2009). However, the incorporation of other supplements such as probiotics, whole yeast (WY) or *Saccharomyces cerevisiae* extract (YE) for meat and carcass quality improvement has been questioned and many unclear results have been shown. According to Kabir (2009), some authors reported advantages of probiotic administration whereas others did not ob-serve improvement when probiotics were used. In their study (Kabir, 2009), supplementation of antibiotics incorporation with probiotics in broiler ration could improve the meat quality both in prefreezing and postfreezing storage. Bacteriocins are other product that can be use. Their antimicrobial activity has been found more effective against strains closely related to the organism that produced them, or against bacteria from the same ecological system (Settanni and Corsetti, 2008). Bacteriocins are ribosomally synthesized cationic peptides with a narrower spectrum of antimicrobial activity than most antibiotics. These are produced by the bacteria that have adapted for competition against other micro-organisms (Nawaz *et al*., 2009).

From the findings of this study, it is evident that there were relatively high incidence of multidrug resistant organisms in poultry feeds tested and relatively low effect of the test antibiotics on these MDR organisms. This has been attributed to either the ability of some of these organisms to produce resistant spores, or poor quality of antibiotics utilized or lesser concentration of antibiotics incorporated, or damage to incorporated antibiotics during feeds manufacturing etc. thereby reducing potency of the antibiotics. These factors could have enhanced the high incidence rate of resistant organisms in poultry feeds sold in Calabar metropolis, Nigeria. However the important role played by the selective pressure should not be neglected as it this condition, which selects only those cells who have adapted to the conditions. The sequential continuous process of mutation and selection gives rise to the highly adapted strains so much so that sometimes the ability to survive in primary environment such as soil or distilled water is lost (Hogardt *et al*., 2007).

Although the number of isolates was relatively small, the level of multidrug resistance was high. The high rates of resistance found in the present study can be explained by the spread of use of antibiotics agents given to poultry in Nigeria as prophylaxis, growth promoters or treatment. According to Abdellah *et al*. (2009), the multiple resistances observed were also to those antimicrobials frequently employed in veterinary practices. Therefore, we recommend that more restrictions on the irrational use of antibiotics and public awareness activities should be undertaken to alert the public to the risks of the unnecessary use of antibiotics. To reduce the effect of these multidrug resistant organisms in poultry feeds; antibiotics incorporated into feeds should be in synergistic combinations, as this will prevent the possibility of resistance development. International trade of food products is expected to increase in the future. Thus, endeavors to improve food safety must take into account the importance of resistant *Salmonella* spp. in imported food products and, through international agreements, limit contamination with antimicrobial drug-resistant *Salmonella* spp. at the primary production site (Skov *et al*., 2007).

CONCLUSION

Our study has demonstrated that these poultry feeds in Calabar, Nigeria are of poor quality (microbiologically) and the contamination is possibly due to poor production, processing and packaging management of the feeds and existence of poor sanitation. The presence of *E. coli* and *Salmonella* sp. in animal (poultry) feeds is of public significance as it is indicative of faecal contamination. Considering that fingers are prone to faecal contamination during toilet use, such practices can easily promote occurrence of diarrhoeal disease outbreaks in humans

through cross-contamination. Environmental organisms such as *Bacillus* spp. and various bacteria of non-medical importance, coming form other sources such as air dust, soil and water add to this collection of MDR organisms found in poultry feeds. Moreover, this implies that drug-resistant poultry-source isolates may originate from some exogenous reservoir (such as raw materials for compounding poultry feeds) later during the packaging of feeds and distribution process, rather than being introduced from the birds. This in turn suggests that on-farm practices, including use of antimicrobial agents for growth promotion, metaphylaxis, and therapy, may influence characteristics of *E. coli* that contaminate retail poultry products and, seemingly, are then transmitted to humans.

This study also reveals that poultry feeds are often contaminated with multi-drug resistant organisms. Occurrence of Multidrug Resistant (MDR) organisms from contaminated water, food substances, animal feeds, chronically infected animals and humans has been a major reason for ultimate failure of antibiotics. Early studies had shown that the environment and the virulence factors are the major reason for the MDR nature of these opportunistic pathogens. However recent investigations have proved that it is the ability of increasing the rate of mutations that allow this organism to adapt to the heterogeneous and dynamic atmosphere of the environment (Seshadri and Chhatbar, 2009). These insights into the survival strategies of these organisms should open ways to newer targets that are susceptible to new methods and allow us to tackle these infections. It heighten concerns regarding the potential human health risk for antimicrobial drug use in poultry feeds, poultry production, and suggest that avoidance of poultry consumption may not reliably provide personal protection. Ongoing national surveillance of Multidrug Resistant (MDR) organisms in humans, livestock, and animal feeds at the retail level and antimicrobial susceptibility testing are necessary to evaluate the effects of implementing European policies. Further research is also needed to better understand the relationship between antimicrobial use in animals and humans and bacterial resistance in humans.

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