ANTI-MICROBIAL RESISTANCE PROFILE OF *Escherichia coli* ISOLATES FROM COMMERCIAL POULTRY FEEDS AND FEED RAW MATERIALS

[1]OKOLI, Ifeanyi Charles., [1]HERBERT, Udoh., [2]OZOH, Patrick and [2]UDEDIBIE, Adive Boniface
[1]Tropical Animal Health and Production Research Laboratory, Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria.
[2]Department of Molecular Biology, Federal University of Technology Owerri, Nigeria.

Corresponding Author: Dr. Okoli, I. C. Tropical Animal Health and Production Research Laboratory, Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria. Email: dr_charleso@yahoo.com

ABSTRACT

Information on the level to which commercial feeds and feed raw materials are involved in the dissemination of anti-microbial resistant pathogenic and commensal bacteria in Nigeria is necessary for feed and stock management. Forty four Escherichia coli isolates from 4 commercial feed brands coded SF, GF, TF and ACF and from 90 various feed raw materials such, fish meal (FM), maize (MA), maize offal (MO), wheat offal (WO), spent grain (SG), blood meal (BM) and soybean meal (SM) etc were screened for anti-microbial resistance profile against 10 antibiotics using the disc diffusion method. Overall, the isolates recorded 80.8 % resistance to cefuroxime, 76.9 % to nalidixic acid, 75 % to ampicillin, and 59.6 % to cotrimoxazole while very low 7.7 % was recorded for tetracycline and 5.8 % for gentamycin, ciprofloxacin and chloramphenicol. Across commercial feed brands, isolates from SF were resistant to nitrofurantoin (100 %), nalidixic acid (50 %) and ampicillin (70 %), while those from TF, GF and ACF were resistant to 7, 6 and 5 antibiotics respectively. Resistance against ampicillin, nalidixic acid and cefuroxime, in isolates from SG, palm kernel cake (PK), MO and WO were high. Organisms isolated from SG and PK recorded high resistance against cefuroxime and cotrimoxazole. Isolates from bone/limestone (B/L) registered 100 % resistance against ampicillin, cotrimoxazole and cefuroxime, while those from maize MA recorded 100 % resistance to cefuroxime and norfloxacin, and over 70 % to nalidixic acid. Soybean meal isolates values for nitrofurantoin, tetracycline, nalidixic acid and ampicillin were high but below 80 %. Thirty fives resistance patterns were observed; with the CF-NB-CO-NA-AM pattern being the most predominant (occurring 10 times). The present data shows that commercial feeds and feed raw materials are important vehicles for the introduction of multi-drug resistance encoding E coli into poultry.

Keywords: Anti-microbial resistance, *E. coli*, commercial feeds, feed raw materials, antibiotics

INTRODUCTION

Evolution and spread of anti-microbial resistant bacterial strains have become a global problem and could possibly be described as a silent epidemic of the millennium (Bush, 1997; OIE, 1999; EMEA, 1999; Franklin *et al*, 2001; Okoli *et al*, 2002 and Stratton, 2003). Anti-microbial resistance, especially of pathogenic bacteria, has been partly attributed to the misuse of anti-microbial agents in medicine and agriculture (van den Bogaard, 1997; Apley *et al*, 1998; Okeke *et al*, 1999 and Okoli *et al*, 2002). The critical importance of this evolution and dissemination of drug resistant bacteria is reflected by the existence of many national and international networks on the surveillance of anti-microbial resistance in bacteria. Thus, there is a need to constantly monitor susceptibility trends in bacterial agents of economic and public health importance.

E coli is an intestinal inhabitant of all animals and is therefore widely distributed (Gross, 1994). They are responsible for numerous animal diseases of economic and public health importance (Jordan, 1990). *E. coli* strains are known to exhibit considerable variations in sensitivity to anti-microbial agents such that isolates from one area of a country or those from different diseases within the same area may vary in sensitivity (Blood and Radostits, 1989). Several materials such as litter, fecal matter, dust in poultry houses, rodent droppings, water and feeds among others have been implicated as possible sources of *E. coli* in poultry (Jordan, 1990).

Of all these possible routes, commercial feeds and feed ingredients are generally regarded as major routes of *E. coli* outside the flock manager's control (Wilson, 1990; Garland, 1996). Since commercial feeds and feed ingredients are usually sourced from wide geographical locations, they remain the major vehicles for the introduction of *E. coli* strains harboring novel resistant factors to local farm environment. Published information on the level to which commercial feeds and feed raw materials are involved in the dissemination of anti-microbial resistant pathogenic and commensal bacteria in Nigeria is however scanty (Uwaezuoke *et al*, 2000). The need to further assess the resistance status of pathogenic and commensal microbes in farm inputs is therefore very imperative and urgent.

The present study determines the prevalence of anti-microbial resistance in *E. coli* isolates from commercial poultry feeds and feed ingredients in Imo State, Nigeria.

MATERIALS AND METHODS

Study Area: The study was carried out in Imo State, Nigeria. Imo State is situated in the central part of the southeastern region of Nigeria. The State is divided into 27 Local Government Areas (LGA) for administrative purposes. These LGAs are further grouped into 3 senatorial zones namely, Owerri, Orlu and Okigwe. Poultry production in the study area could be broadly divided into extensive, semi-intensive and intensive systems. The greatest populations of chicken in the study area were made of local breeds reared by rural farmer under the extensive system. Other poultry producers included the owners of small to medium scale poultry farms that are sited in both rural and urban areas (Sonaiya, 2000). Rearing of started exotic broilers and cockerels has also become an important aspect of this production system in Imo State (Meremikwu, 2001).

Commercial intensive productions include table eggs, broiler, parent stock, turkey and chicken. These operations have been shown to range from very small scale (50 - 100 birds), to medium scale (101 to 1000 birds) and large scale (above 1000 birds). Small and medium farms are usually back yard affairs predominantly found in urban and peri-urban centers. Large scale, operations are located in peri-urban and rural environments. In most of the back yard poultries, hygienic and bio-security measures were usually poor with all the family members being involved in the daily management activities; usually there was no organized effort at vermin, ferrets and human traffic control. Hatcheries located in the area were involved in in-house and toll hatching.

Commercial poultry farmers in the area usually purchase their feeds from dealers on any of the popular commercial feed brands such as Top, Sanders, Guinea, Livestock and Vital Feeds among others. Most large-scale operators produce their own feeds from feed raw materials like fish meal, maize, maize offal, wheat offal, spent grain, blood meal and soybean meal etc purchased from dealers. Water is obtained from public taps where available or from streams or harvested rainwater. Self-medication was very rampant among the farmers with some of them also using human drugs for the treatment of poultry diseases (Okoli *et al*, 2002).

Sample Collection: Four popular commercial feeds and 2 feed raw materials depots selling common brands of feeds that farmers usually buy and assorted feed raw materials were purposively selected for the study. The four commercial feed brands sampled were coded SF, GF, TF and ACF. Feed raw materials sampled included, fish meal, maize, maize offal, wheat offal, spent grain, blood meal and soybean meal. Each commercial feeds and feed raw material depot was visited twice over a period of 6 weeks for sampling.

Samples were collected to cover the different types of poultry feed such as layer mash, chick mash, broiler starter mash, broiler finisher mash, grower mash and breeder mash. Three bags of feed were randomly sampled for each type of poultry mash, after which they were pooled to make a representative sample of that type of mash for a particular feed brand. Sampling was done by carefully opening each selected bag of feed and collecting approximately 5 grams of the feed with sterile universal bottles. Thereafter, the brand name, type of mash and town produced were recorded. The same procedure was adopted in sampling the feed raw materials. Sixty-two commercial poultry feeds and 110 feed raw materials were sampled.

Cultivation and Isolation of Organisms: Five grams of each feed sample was properly homogenized in 45 ml of sterile water and a 10-fold serial dilution of the homogenized samples was done (Ogbulie and Okpokwasili, 1999). A 0.1 ml aliquot of the appropriate dilution was inoculated onto MacConkey agar (MCA) plates. In all cases, the inoculation techniques described by Cruickshank *et al* (1983), were adopted. The plates were incubated overnight at 37 ^{0}C.

Growths on the MCA plates suggestive of *E. coli* colonies, 2 – 4 mm in diameter, opaque and convex edge and rose pink colonies on account of lactose fermentation (Gillies and Dodds, 1976) were further isolated onto eosin methylene blue (EMB) plates and incubated overnight at 37 ^{0}C. Green metallic sheen colonies indicative of *E. coli* were then subjected to biochemical tests for *E. coli* identification (Edwards and Ewing 1972).

Susceptibility Testing: The isolated *E. coli* were screened for anti-microbial resistance profile using the disc diffusion method (Bauer *et al,* 1966) as recommended by the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). The disc diffusion method is widely recognized to work well with rapidly growing facultatively anaerobic and aerobic organisms such as Enterobacteriaceae (NCCLS, 1999).

Commercial antibiotics drugs used in the study included AM, ampicillin (25 µg); CO, cotrimoxazole (50 µg); NI, nitrofurantoin (100 µg); GN, gentamycin (10 µg); NA, nalidixic acid (30 µg); TE, tetracycline (30 µg); CH, chloramphenicol (10 µg); CF, cefuroxime (20 µg); NB, norfloxacin (10 µg); CP, ciprofloxacin (5 µg).

Data Collection and Interpretations: Susceptibility data were recorded quantitatively by measuring the diameters to the nearest millimeter using a meter rule. Using the interpretative chat of Kirby-Bauer Sensitivity Test Method (Cheesbrough, 2000), the zones were interpreted as resistant or sensitive. For the purpose of the present study, isolates with intermediate sensitivity were categorized as sensitive. Furthermore, proportions of isolates resistant to individual drugs and having each anti-microbial resistance patterns were computed according to feed brands and raw material types.

RESULTS

Feed Sampling, Isolation and Cultivation of Organisms: Forty four (70.97 %) *E. coli* organisms were isolated from the 62 commercial feeds sampled and 90 (81.82 %) *E. coli* were isolated from raw materials. Table 1 showed that most of the sampled commercial feeds and feed raw materials originated from outside the state. Fish meal, maize and ACF for example originated from places located more than 500 kilometers from the sampling sites.

Table 1: Sampling sites, town of production and estimated distance to sampling sites of poultry feeds and feed raw materials

Sample type	Sampling site	Town of Production	Estimated distance from sampling site (Km)
(A) Commercial feeds			
ACF	Owerri	Lagos	500+
SF	Owerri	Aba	40
TF	Owerri	Sapele	150
GF	Owerri	Ewu	250+
(B) Feed raw materials			
Fish meal (FM)	Owerri	Maiduguri	1200+
Maize (MA)	Owerri	Jos	800+
Palm kernel cake (PK)	Owerri	Enugu	150
Spent grain (SG)	Owerri	Aba	40
Wheat offal (WO)	Owerri	Port Harcourt	80
Maize offal (MO)	Owerri	Aba	40
Limestone (LS)	Owerri	Calabar	180
Bone meal (BO)	Owerri	Mbaise	20
Blood meal (BM)	Owerri	Mbaise	20
Soybean meal	Owerri	Enugu	150

The overall anti-microbial resistance frequencies of *E. coli* isolates from commercial feeds and feed raw materials are presented in Figure 1.

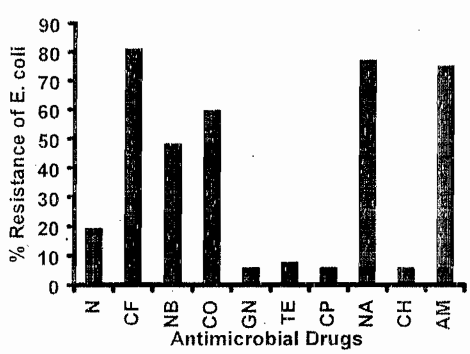

Figure 1: Overall anti-microbial resistance frequencies of E coli isolates from commercial feed brands and feed ingredients sold in Imo state. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamycin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

The isolates were 80.8 % resistant to cefuroxime, 76.9 % to nalidixic acid, 75.0 % to ampicillin, and 59.6 % to cotrimoxazole. Very low resistances (5.8 %) were recorded for gentamycin, ciprofloxacin and chloramphenicol, while 7.7 % was returned for tetracycline.

Anti-microbial Resistance Profile of *E. coli Isolates* from Commercial Feeds: Across commercial feed brands (Figure 2), isolates from SF were resistant to only nitrofurantoin (100.0 %), nalidixic acid (50.0 %) and ampicillin (70.0 %). Organisms isolated from TF and GF on the other hand were resistant to 7 and 6 antibiotics respectively, while those from ACF were resistant to 5 with values for these remaining below 100.0 %. None of the isolates recorded resistance against ciprofloxacin and chloramphenicol, while those from GF and TF recorded very low rates to gentamycin and tetracycline.

Table 2, showed that *E. coli* isolates from SF had significantly higher resistance against nitrofurantoin and ampicillin ($P < 0.05$), while with the exception of nalidixic acid, the other antibiotics had low resistances. Resistance figure for nalidixic acid among ACF isolates was also significantly low while GF values recorded for cefuroxime, norfloxacin, cotrimoxazole and gentamycin were significantly higher than that of the others ($P < 0.05$).

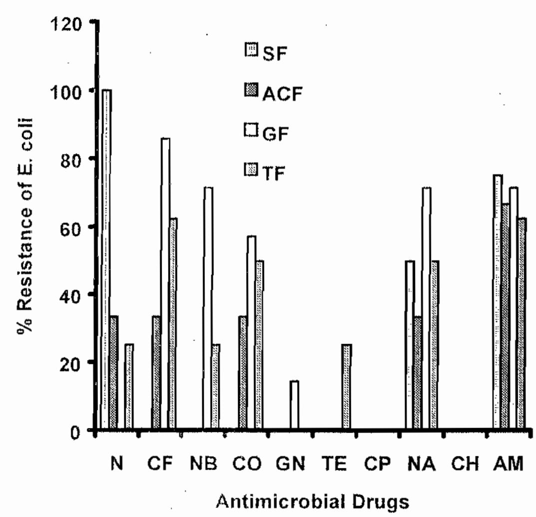

Figure 2: Comparison of anti-microbial resistance frequencies of E coli isolates from commercial feed brands sold in Imo state. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamycin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin. SF = Sanders feed; ACF = Animal care feed; GF = Guinea feed; TF = Top feed.

Table 2: Anti-microbial resistance frequencies of *E coli* isolates from commercial feed brands sold in Imo State

Feed Type	NI	CF	NB	CO	GN	TE	CP	NA	CH	AM	n
SF	8(100.0)^{a}	0(0.0)^{b}	0(0.0)^{b}	0(0.0)^{b}	0(0.0)^{b}	0(0.0)^{b}	0(0.0)	4(50.0)^{b}	0(0.0)	6(75.0)^{a}	8
ACF	2(33.3)^{a}	2(33.3)^{ab}	0(0.0)^{b}	2(33.3)^{ab}	0(0.0)^{b}	0(0.0)^{b}	0(0.0)	2(33.3)^{b}	0(0.0)	4(66.7)^{b}	6
GF	0(0.0)^{a}	12(85.7)^{a}	10(71.4)^{a}	8(57.2)^{a}	2(14.3)^{a}	0(0.0)^{b}	0(0.0)	10(71.4)^{a}	0(0.0)	10(71.4)^{ab}	14
TF	4(25.0)^{a}	10(62.5)^{a}	4(25.0)^{ab}	8(50.0)^{a}	0(0.0)^{b}	4(25.0)^{a}	0(0.0)	8(50.0)^{b}	0(0.0)	10(62.5)^{b}	16
Total	14(31.8)	24(54.6)	14(31.8)	18(40.9)	2(4.6)	4(9.1)	0(0.0)	24(54.6)	0(0.0)	30(68.2)	44
SEM	21.3	18.5	16.8	12.7	3.6	6.3	0.0	7.8	0.0	2.7	

ab means with different superscripts in the same column are significantly different (P<0.05). N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamycin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

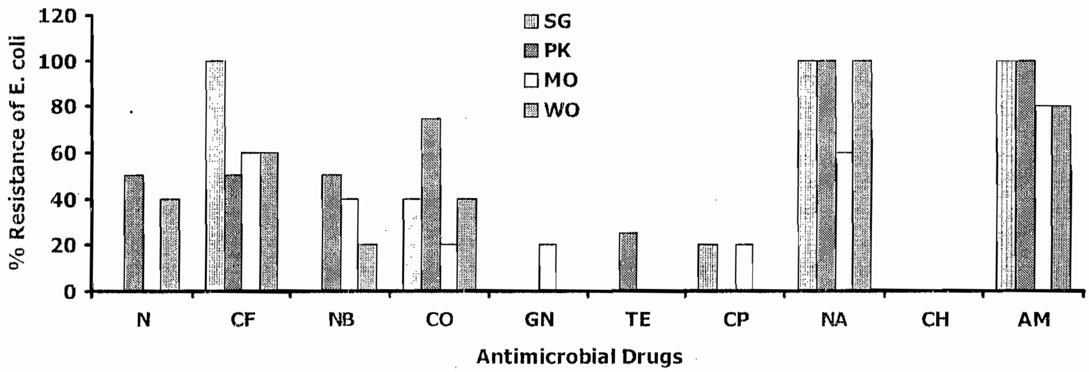

Figure 3: Anti-microbial resistance frequencies of E coli isolates from different feed ingredients sold in Imo state. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamycin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin. SG = Spent grain; PK = Palm kernel cake; MO = Maize offal; WO = Wheat offal.

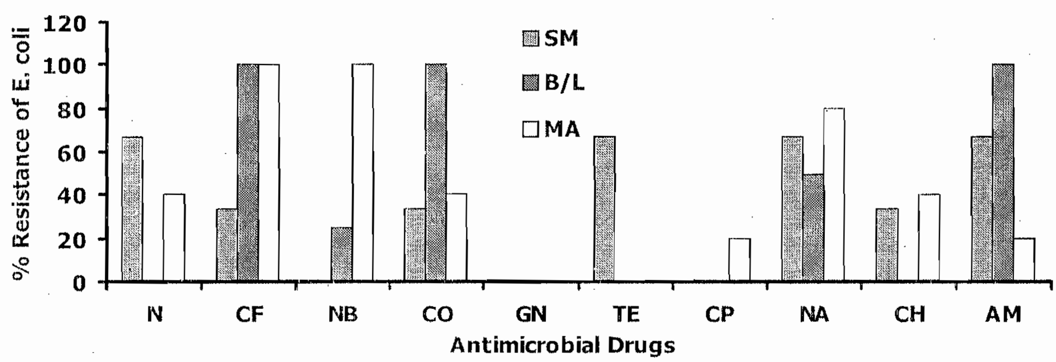

Figure 4: Anti-microbial resistance frequencies of E. coli isolates from soybean meal (SM), bone/limestone (B/L) and maize (MA) sold in Imo state. N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamycin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin. SM = Soybean meal; B/L = Bone meal/ Limestone; MA = Maize.

Anti-microbial Resistance Profile *E. coli* of Isolates from Feed Raw Materials: Anti-microbial resistance of *E coli* isolated from various feed raw materials is presented in Figure 3. Values recorded against ampicillin, nalidixic acid and cefuroxime, in isolates from spent grain (SG), palm kernel cake (PK), maize offal (MO) and wheat offal (WO) were relatively high.

Organisms isolated from SG and PK recorded high resistance against cefuroxime and cotrimoxazole respectively. No resistance was observed against chloramphenicol in any of the sample types, while resistance to gentamycin and tetracycline were observed exclusively in MO and PK isolates respectively.

Table 3: Anti-microbial resistance frequencies of *E. coli* isolates from different feed raw materials sold in Imo State

FEED TYPE	N	CF	NB	CO	GN	TE	CP	NA	CH	AM	n
Spent grain	0(0.0)^{c}	10(100.0)^{a}	0(0.0)^{c}	4(40.0)^{bc}	0(0.0)^{b}	0(0.0)^{c}	2(20.0)^{a}	10(100.0)^{a}	0(0.0)^{b}	10(100.0)^{a}	10
Palm kernel cake	4(50.0)^{b}	4(50.0)^{bc}	4(50.0)^{b}	6(75.0)^{a}	0(0.0)^{b}	2(25.0)^{b}	0(0.0)^{b}	10(100.0)^{a}	0(0.0)^{b}	10(100.0)^{a}	8
Maize offal	0(0.0)^{c}	6(60.0)^{b}	4(40.0)^{b}	2(20.0)^{c}	2(20.0)^{a}	0(0.0)^{b}	2(20.0)^{a}	6(60.0)^{c}	0(0.0)^{b}	8(80.0)^{ab}	10
Wheat offal	4(40.0)^{b}	6(60.0)^{b}	2(20.0)^{c}	4(40.0)^{bc}	0(0.0)^{b}	0(0.0)^{c}	0(0.0)^{b}	10(100.0)^{a}	0(0.0)^{b}	8(80.0)^{ab}	10
Fish meal (No)	2(14.3)^{c}	14(100.0)^{a}	12(85.7)^{ab}	14(100.0)^{a}	0(0.0)^{b}	0(0.0)^{c}	0(0.0)^{b}	14(100.0)^{a}	0(0.0)^{b}	12(85.7)^{ab}	14
Fish meal (On)	4(40.0)^{b}	8(80.0)^{ab}	6(60.0)^{b}	4(40.0)^{bc}	0(0.0)^{b}	2(20.0)^{bc}	0(0.0)^{b}	8(80.0)^{b}	0(0.0)^{b}	6(60.0)^{b}	10
Soybean meal	4(66.7)^{a}	2(33.3)^{c}	0(0.0)^{c}	2(33.3)^{bc}	0(0.0)^{b}	4(66.7)^{a}	0(0.0)^{b}	4(66.7)^{bc}	2(33.3)^{a}	4(66.7)^{b}	6
Bone/limestone	0(0.0)^{c}	8(100.0)^{a}	2(25.0)^{c}	8(100.0)^{a}	0(0.0)^{b}	0(0.0)^{c}	0(0.0)^{b}	4(50.0)^{c}	0(0.0)^{b}	8(100.0)^{a}	8
Maize	4(40.0)^{b}	10(100.0)^{a}	10(100.0)^{a}	4(40.0)^{bc}	0(0.0)^{b}	0(0.0)^{c}	2(20.0)^{a}	8(80.0)^{b}	4(40.0)^{a}	2(20.0)^{c}	10
Total	**22(25.6)**	**68(79.1)**	**40(46.5)**	**48(55.8)**	**2(2.3)**	**8(9.3)**	**6(7.0)**	**74(86.1)**	**6(7.0)**	**68(79.1)**	**86**
SEM	**8.3**	**8.6**	**11.8**	**9.9**	**2.2**	**7.5**	**3.3**	**6.5**	**5.4**	**8.6**	

abc means with different superscripts in the same column are significantly different (P<0.05). N, nitrofurantoin; Cf, cefuroxime; Nb, norfloxacin; Co, cotrimoxazole; Gn, gentamycin; Te, tetracycline; Cp, ciprofloxacin; Na, nalidixic acid; Ch, chloramphenicol; Am, ampicillin.

Table 4: Anti-microbial resistance patterns of *E coli* isolates from different commercial feeds and feed raw materials sold in Imo state

Resistance pattern	Freq. of occurrence	Resistance pattern	Freq. of occurrence
I Zero Resistance	4	2 NA	1
3 NI	1	4 NA-AM	3
5 CF-AM	1	6 CF-NB	1
7 CF-NA	1	8 NI-NA	2
9 NI-AM	3	**10 CF- CO**	1
11 CF-CO-AM	6	12 CF-NA-AM	5
13 CF-CO-NA	1	14 CF-NB-NA	1
15 CF-NB-AM	1	16 NB-NA-AM	1
17 CF-CO- NA-AM	2	**18 CF-CP-NA-AM**	1
19 CO-TE-NA-AM	2	20 CF-NB-NA-CH	1
21 NI-CF-NB-NA	1	22 NB-CO-GN-NA-AM	1
23 CF-NB-CP-NA-AM	1	24 NI-CF-CO-NA-AM	1
25 CF-NB-CO-NA-AM	10	26 NI-CF-NB-CO-NA	1
27 CF-CO-TE-NA-AM	1	28 NI-CF-TE-NA-AM	1
29 NI-CF-NB-CO-NA-AM	4	30 CF-NB-CO-GN-NA-AM	1
31 NI-CO-TE-NA-CH-AM	1	32 CF-NB-CO-TE-NA-AM	1
33 NI-CF-NB-CO-GN-CH-AM	1	34 NI-CF-NB-CO-CP-NA-CH	1
35 NI-CF-NB-CO-TE-NA-AM	1		

N, nitrofurantoin; CF, cefuroxime; NB, norfloxacin; CO, cotrimoxazole; GN, gentamycin; TE, tetracycline; CP, ciprofloxacin; NA, nalidixic acid; CH, chloramphenicol; AM, ampicillin.

Figure 4 showed the anti-microbial resistance frequencies of *E. coli* isolates from soybean meal (SM), bone/limestone (B/L) and maize (MA). Isolates from bone meal and limestone were 100 % resistant against ampicillin, cotrimoxazole and cefuroxime, while those from maize recorded 100 % resistance to cefuroxime and norfloxacin, and over 70 % to nalidixic acid. Soybean meal isolates values for nitrofurantoin, tetracycline, nalidixic acid and ampicillin were high but below 80 %. None of the isolates from B/L, MA and SM recorded resistance against gentamycin.

Table 3 showed that isolates from spent grain and palm kernel cake had significantly high resistance to cefuroxime, nalidixic acid, and ampicillin (P < 0.05), while wheat offal and fish meal also had significantly high resistance to nalidixic acid and cefuroxime (P < 0.05). Isolates from bone/limestone recorded significantly (P < 0.05) higher resistance to cefuroxime, cotrimoxazole and ampicillin, while those from maize on the other hand, equally returned significantly higher resistance to cefuroxime and norfloxacin. Ciprofloxacin values in isolates from spent grain and maize offal, gentamycin value in those from maize offal, tetracycline and chloramphenicol values from soybean meal isolates, were all significantly (P < 0.05) higher than figures returned for others.

Anti-microbial Resistance Patterns: Anti-microbial resistance patterns of *E. coli* isolates from different commercial feeds and feed materials are presented in Table 4. Thirty fives resistance patterns were observed, with CF-NB-CO-NA-AM pattern being the most predominant and occurring 10 times. The pattern was observed in isolates from fish meal, blood meal, GF and TF. Other resistance patterns, CF-CO-AM and CF- NA-AM occurred 6 and 5 times respectively. CF-CO-AM was observed in GF, and TF, spent grain, limestone and blood meal, CF- NA-AM was observed in isolates from spent grain, maize offal, wheat offal and fish meal. Organisms recording zero resistance occurred 4 times and were predominant in maize offal, TF and soybean meal. The NI-CF-TE-NB-CO-NA-AM pattern equally occurred 4 times and were observed in palm kernel cake, wheat offal and fish meal. Sixteen of the resistance patterns contained between 0 and 3 antibiotics per pattern, while the rest 19 contained between 4 and 7 antibiotics.

DISCUSSION

Overall, *E. coli* isolates from commercial feeds and feed raw materials had very high resistance to cefuroxime, nalidixic acid, ampicillin, and cotrimoxazole, while the rate against norfloxacin was moderate at 48.1 %. Low resistant rates (5.8 %) were recorded for gentamycin, ciprofloxacin and chloramphenicol. 7.7 % for tetracycline and 19.2 % against nitrofurantoin were also recorded. The present result does not agree with the over 90 % resistant rate reported by Uwaezuoke *et al* (2000) for gentamycin in *E. coli* isolates from a cocktail of feeds obtained from Owerri. Commercial feeds and feed ingredients are generally regarded as major routes of coliform and bacterial infections in poultry (Wilson, 1990; Garland, 1996).

Isolates from GF, a commercial feed brands, harbored *E. coli* organisms with relatively higher resistance against cefuroxime, norfloxacin, cotrimoxazole, nalidixic acid and ampicillin, while those from SF harbored *E. coli* resistant against nitrofurantoin, nalidixic acid and ampicillin. It would therefore seem from the present study that GF might be a major vehicle for the dissemination of resistant bacteria in the study area. None of the commercial feed brands was implicated in the dissemination of ciprofloxacin and chloramphenicol resistant *E. coli.*

Feed raw materials were found to harbor preponderantly more resistant bacteria than the commercial feeds. Fish meal and maize were specifically important vehicles for the dissemination of quinolone resistant *E. coli* isolates. The fish meal from Maiduguri returned between 60 and 100 % resistance to norfloxacin and nalidixic acid. These isolates also recorded very high values against ampicillin, cotrimoxazole and cefuroxime but 0% resistance to ciprofloxacin, tetracycline and gentamycin. Of interest also is the 100 % resistance recorded in isolates from maize to norfloxacin and cefuroxime. Since these drugs are not readily employed in veterinary therapy, the present data suggests that the major source of these organisms might be through human contamination during the processing stages of the feed raw materials. The high overall resistance rates in the *E. coli* isolates from the present study may thus be attributed to the fact that *E. coli* occupies multiple niches in the environment including human and animal hosts and can in addition exchange genetic materials with many other bacteria (Blood and Radostits, 1989).

Thirty-five resistance patterns were observed; with the CF-NB-CO-NA-AM pattern being the most predominant and occurring 10 times. The pattern was well distributed in isolates from fish meal sourced from Maiduguri, blood meal from Mbise, GF from Ewu and TF from Sapele. Sixteen of the resistance patterns contained between 0 and 3 antibiotics per pattern, while the rest 19 contained between 4 and 7 antibiotics. The present study shows that the most important factor in the dissemination of resistance factors through commercial feeds and feed ingredients is the fact that they are sourced from a very wide area. Thus, organisms from Maiduguri in northern Nigeria or those from Lagos in the west may end up in a small village farm in Imo State and become the focus for the establishment of new resistance mechanisms in the area. While different treatments including heating, acidification (Nape and Murphy, 1971; Cox *et al,* 1986) and other forms of treatment (Nielsen, 1992; Haggblom 1993; Dorey, 2001) have been found relatively effective in reducing bacterial load in feeds, such methods should be easily adopted in a developing economy such as Nigeria in order to reduce E. coli buildup in commercial feed.

ACKNOWLEDGEMENT

This work was financed from the Federal University of Technology Senate grant No. 4701578(11-757-4260). We are grateful to Dr. K. F. Chah of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka for laboratory analysis of the feedstuff.

REFERENCES

APLEY, M. D., BROWN, S. A., FEDORKA-CRAY, P. J. and FERENCE, S. (1998). Role of veterinary therapeutics in bacterial resistance development: animal and public health perspectives. *Journal of American Veterinary Medical Association, 212:* 1209 – 1213.

BAUER, A. W., KIRBY, W. M. M., SHERRIS, J. C. and TURCK, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American of Journal Clinical Pathology, 36:* 493 – 496.

BLOOD, D. C. and RADOSTITS, O. M. (1989). *Veterinary medicine.* 7th Edition, Bailliere and Tindall, London.

BUSH, K. (1997). The evolution of ß-lactamases. Pages 152 – 166. *In: Antibiotic resistance: origin evolution, selection and spread.* John Wiley, New York.

CHEESBROUGH, M. (2000). Microbiological Tests. *In: District laboratory practice in tropical countries.* Part 2. Cambridge University Press, Cambridge.

COX, N. A., BURDICK, D., BAILEY, J. S. and THOMSON J. E. (1986). Effect of steam conditioning and pelleting process on the microbiological and quality of commercial type poultry feeds. *Poultry Science, 65:* 704 – 709.

CRUICKSHANK, R., DUGUID, J. P., MARMION, B. P. and SWAIN, R. H. A. (1983). *Medical microbiology.* Volume 2, 12th edition, Churchill Livingstone, Edinburgh.

DOREY, L. (2001). Salmonella free chickens: From farm to fork. *In Focus, 25(3):* 6 – 7.

EDWARDS, P. R. D. and EWING, W. R. (1972). *Identification of Enterobacteriaceae.* 3rd edition, Burgess Publishing Company, Minneapolis, Minnesota.

EMEA (1999). Antibiotic resistance in the European Union associated with therapeutic use of veterinary medicines. *Proceedings of European Meeting on Antibiotics,* 14th July 1999, London. Pp. 251 – 259.

FRANKLIN, A. ACAR, J., ANTHONY, F., GUPTA, R., NICHOLLS, T., TAMURA, Y., THOMPSON, S.,

THRELFALL, E. J., VOSE, D., VAN VUUREN, M., WHITE, D. G., WEGENER, H. C., and COSTARRICA, M. L. (2001). Anti-microbial resistance: harmonization of national anti-microbial resistance monitoring and surveillance programmes in animals and in animal derived food. *Review de Science Technique Office Internationale Epizootics, 20(3):* 859 – 870.

GARLAND, P. W. (1996). *Salmonella prevention and salmonella inhibitors in poultry feed.* Technical Publication of BOCM, Pauls Limited, Ipswich. 28 pp.

GILLIES, R. R. and DODDS, T. C. (1976). *Bacteriology illustrated* 4th edition. Churchill Livingstone Edinburgh, London. 156 pp.

GROSS, W. B. (1994). Diseases due to *Escherichia coli* strains in poultry. Pages 237 – 259. *In:* GYLES, C. I. (Ed.). *Escherichia coli in domestic animals and humans*: CAB International, Wallingford, U.K. Pp.

HAGGBLOM, P. (1993). Monitoring and control of salmonella in animal feed. Pages 127 – 137. *In: Proceedings of International course on salmonella control in animal production and products.* National Veterinary Institute, Uppsala, Sweden. August 21 – 27, 1993.

JORDAN, F T W (1990). *Poultry diseases.* 3rd ed. Bailliere and Tindall, London. 231 pp.

MEREMIKWU, V. N. (2001). *The performance of started broilers under subsistence free-range system of production in Nigeria.* MSc. Thesis, Federal University of Technology Owerri, Nigeria. 128 pp.

NCCLS (1999). Performance standard of anti-microbial disk and dilution susceptibility tests for bacteria isolated from animals. *National Committee for Clinical Laboratory Standards, Approved standards,* M 31 – A, 19 (11).

NIELSEN, B. B. (1992). Production of Salmonella free poultry feed. Pages 436 – 441. *In: International Symposium on Salmonella and Salmonellosis.* Ploufragan, France.

NAPE, W. F. and MURPHY, C. (1971). Cited in HOFSTAD, M. S., CALNECK, B. W., HELMBOLDT C. F., REID, W. M. and YODER, H. W. Jr. (eds.) (1978). *Diseases of poultry* 7th Edition, Iowa State University Press, Ames, Iowa, USA.

OGBULIE, J. N. and OKPOKWASILI, G. C. (1999). Efficacy of chemotherapeutic agents in controlling bacterial diseases of cultured fish. *Journal Aquaculture in the Tropics, 13:* 61 – 72.

OIE, (1999). The use of antibiotics in animals ensuring the protection of public health. Pages 68 – 91. *In: Proceeding of European Scientific Conference of the Office Internationale de Epizootics (OIE). Regional Commission of Europe, Paris.* 24 – 26 March, 1999.

OKEKE, I. N., LAMIKANRA, A. and EDELMAN, R. (1999). Socio-economic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases, 5(1):* 13 – 27.

OKOLI, I. C., NWOSU, C. I., OKOLI, G. C., OKEUDE, N. J. and IBEKWE, V. (2002) Drug management of anti-microbial resistance in avian bacterial pathogens in Nigerian. *International Journal of Environmental Health and Human. Development, 3(1):* 39 – 48.

SONAIYA, E. B. (2000). Family poultry and food security research requirements in science, technology and socioeconomics. *In: Proceedings of the XXI World's Poultry Congress,* Montreal, Canada. August 20 – 24, 2000. (CD ROM).

STRATTON, C. W. (2003). Dead bugs don't mutate: Susceptibility issues in the emergence of bacterial resistances. *Emerging Infectious Diseases, 9(1):* 10-16.

UWAEZUOKE, J. C., OGBULIE, J. N., NJOKU, J. N., OBIAJURU, I. O. C. AND NJOKU, A. J. (2000). Antibiotic sensitivity patterns of bacterial isolates from poultry feed. *International Journal of Environmental Health and Human Development, 1(2):* 23 – 28.

VAN DEN BOGAARD, A. E. (1997). Anti-microbial resistance-relation to human and animal exposure to antibiotics. *Journal of Antimicrobial Chemotherapy, 40:* 453 – 454.

WILSON, S. (1990). Raw materials – Animal proteins. *Proceedings of the Society of Feed Technologists, 1990 meeting on the control of salmonellas,* UK.