

*Full Length Research Paper*

# Distribution, antibiogram and multidrug resistance in Enterobacteriaceae from commercial poultry feeds in Nigeria

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The increasing incidence of enterobacteriosis in poultry birds in Nigeria lately necessitated the study which aimed at enumerating the Enterobacteriaceae in 58 commercially available poultry feeds, studying their antibiotic susceptibility pattern, confirming plasmid as the determinants of resistance and tracing the relationship between multidrug resistant (MDR) isolates in the feed and those in morbid birds. Fifty-three enterobacteria belonging to 5 genera: *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Yersinia*; were obtained from the feed samples. *Escherichia coli* was the most distributed enterobacteria in the feed types (80%). There was a significant ( $P < 0.05$ ) trend in overall percentage distribution of contaminating enterobacteria across feed types in the order: chick mash > broiler starter > broiler finisher > growers mash > layers mash. Augmentin, nitrofurantoin and amoxicillin showed the least overall potency (<30%) against all isolates while sensitivity to fluoroquinolones was above 70%. Multidrug (MD) resistance was limited to 32.1% of the isolates. The plasmid DNA profiles showed that 71.4% of the analyzed MDR strains possessed extrachromosomal determinants with relative sizes of 6.4 to 23.0 kb. The similarity studies showed that there was a direct relationship between MDR *E. coli* strains from the feed samples and those from morbid birds ( $S_i = 78.9\%$ ).

**Key words:** Enterobacteriaceae, poultry feed, antibiogram, multidrug resistance, plasmid.

## INTRODUCTION

Commercial poultry farming has become a major source of income, as well as animal protein to many Nigerians. Birds like chicken, turkeys, ducks, guinea fowl, etc, are domesticated as a source of egg and/or meat production for human consumption. This necessitates the need for poultry feed production on commercial level (Microlivestock, 1991). Poultry feeds are food materials designed to contain all the nutritional materials needed 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.

The Enterobacteriaceae are a large group of related bacteria living in soil, water and decaying matter, and are also common occupants of both human and animal's large bowel. They are acquired through contaminated food or water, and are the major cause of diarrheal illnesses (Talaro and Talaro, 2002). Poultry feeds can be contaminated directly or indirectly through contact with soil, rodents, birds, dust, human carriers, sewage or water during processing and storage. In microbiological analyses of foods, members of the enterobacteriaceae commonly serve as indicators of faecal contamination and they consist of important zoonotic bacteria such as *Salmonella* spp., *Yersinia* spp. and *Escherichia coli* (Miranda et al., 2008). Obi and Ozugbo (2007) recorded that *Salmonella* paratyphoid fever in humans have been associated with the consumption of poultry birds that contracted the infection from contaminated poultry feeds.

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Previously, the incorporation of antibiotics such as bacitracin, tetracycline, oxy- and chlor-tetracycline into poultry feed formulations at low (prophylactic) level, was aimed at helping to prevent minor diseases of this bacteria family, and to enhance efficient growth of the bird (Smith, 2005). However, with the emergence and continuing presence of multi-drug resistant isolates which has aroused so much concern in terms of horizontal transfer of resistant gene, there has been a ban placed on use of antibiotics in poultry feed formulation in the US, Europe and Australia (Paterson, 2006; Cox and Pavic, 2010). Today, poultry feeds still remain a source of various bacteria that can be pathogenic to both human and animal health.

A common health quotation says, "You are what you eat". Currently, it has been established that several antimicrobial-resistant bacteria obtained from humans have their primary origin as animals raised for human consumption (Aarestrup, 2000) and that these bacteria may contaminate the products derived from these animals (Saenz et al., 2001). In Nigeria, there has been few documented studies on the contaminating enterobacteria of poultry feeds and their antibiotics susceptibility patterns (Uwaezuoke et al., 2000; Obi and Ozugbo, 2007). However, the incidence of enterobacteriosis in poultry farms in Nigeria has been on the rise and warrants attention. We could not lay hands on any data from Nigeria for the relationship between bacteria or multi-drug resistant strains from poultry feeds and those from morbid birds. Therefore, with poultry feed at the start of the food safety chain in the 'farm-to-fork' model, it being a possible source of antimicrobial resistant bacteria present in poultry meat (Da Costa et al., 2007), and the need for excellent microbiological safety regulations to escape microbial contamination of the feeds, this study was aimed at enumerating the presence of Enterobacteriaceae in commercially available poultry feeds, studying their antibiotic susceptibility pattern and confirming plasmid as the determinants of resistance in the multidrug resistant (MDR) isolates obtained. The similarity relationship between MDR isolates in the feed and those in morbid birds was also studied.

## MATERIALS AND METHODS

### Sample collection

Fifty-eight commercially available poultry feed samples were collected from 19 districts within 17 states in Nigeria and grouped into 5 categories: Chick mash, growers mash, layers mash, broiler starter and broiler finisher. The producers of the various feed samples remain undisclosed. The districts from where samples were collected include Abakpa, Aba, Benin, Birnin Kebbi, Damaturu, Ijebu-Ode, Ikorodu, Jalingo, Kaduna, Kano, Makurdi, Minna, Mushin, Ogere, Onitsha, Owerri, Port-Harcourt, Warri, Yenegoa. A sixth category, layers premixed, having only a single sample recorded no growth of enterobacteria after repeated isolations. For ease of transportation and aseptic techniques,

samples were sealed in zip-lock bags, transported to the Microbiology Laboratory II of Babcock University and stored at 4°C till further analysis.

### Enterobacteriological analysis

#### *Isolation of contaminating enterobacteria*

The contaminating enterobacteria in the samples were determined considering the ISO Standard 7402 (1993) for Enterobacteriaceae plate count. Each feed sample was serially diluted in 2% sterile peptone water and the  $10^{-3}$  and  $10^{-5}$  dilutions were pour plated in duplicates on Nutrient, Eosin-Methylene Blue, Salmonella-Shigella, and MacConkey agars, all Oxoid products. Each colony was picked and subcultured twice on Eosin-Methylene Blue, Salmonella-Shigella, and MacConkey agars to ascertain consistency in morphological characteristics. Plates were incubated at 37°C for 24 to 48 h. Each morphologically consistent colony was subjected to biochemical characterization according to Cowan and Stell (1993). *Yersinia* was confirmed by subculturing presumptive colonies onto Brain-Heart Infusion agar 7115C (Acumedia, Neogen Inc., MI) with low calcium concentration according to Bhaduri et al. (1997). *Yersinia* and *Enterobacter* were identified only up to the genus level.

#### *Antimicrobial susceptibility test (AST)*

The antimicrobial susceptibility test for each isolate was performed on freshly prepared, dry surfaced Mueller Hinton agar (Oxoid) using the agar-disk diffusion method (CLSI, formerly NCCLS, 2002). The tested antibiotics included: augmentin (30 µg), ceftriaxone (30 µg), nitrofurantoin (200 µg), gentamycin (10 µg), cotrimoxazole (25 µg), ofloxacin (5 µg), amoxicillin (25 µg), ciprofloxacin (10 µg), tetracycline (30 µg) and pefloxacin (5 µg). *E. coli* ATCC 25922 was used as quality control for the test. Based on zones of inhibition, interpreted using the criteria recommended for Enterobacteriaceae by NCCLS (2002), isolates were classified as either sensitive (S) or resistant (R). Isolates resistant to four or more antibiotics were classified as MDR strains.

#### *Molecular studies on MDR strains*

Plasmid profiling of MDR strains: Plasmid profiles of 14 MDR strains were determined by the TENS-Mini Prep method followed by band separation on horizontal gel electrophoresis in 0.8% agarose in 1X Tris-Borate-EDTA (TBE) buffer at room temperature (Lech and Brent, 1987; Kraft et al., 1988). Briefly, single purified bacterial colonies were seeded each into 10 ml Mueller-Hinton broth (Difco) in screw cap tubes and incubated overnight at 37°C. After centrifugation of 1.5 ml of the overnight culture for 1 min, the pelleted cells were dissolved in 300 µl of TENS solution (Tris 25 mM, EDTA 10 mM, NaOH 0.1 N and SDS 0.5%), the tube inverted a few times for thorough mixing and iced for 5 min. An addition of 150 µl of 3.0 M sodium acetate (pH 5.2) was made and the tube vortexed till completely mixed. The solution was micro-centrifuged for 5 min at 13,000 rpm to pellet cell debris and chromosomal DNA. Supernatant (400 µl) was decanted into fresh Eppendorf tube, mixed with 800 µl ice-cold absolute ethanol, and centrifuged for 10 min to pellet the plasmid DNA. The supernatant was discarded, pellet rinsed twice in 1 ml of 70% ice-cold ethanol and dried at 45°C for 15 min. The dried pellet was re-suspended in 40 µl TE buffer and stored at 4°C till further analysis.

For the separation of plasmid DNA, a horizontal tank loaded with 5 mm agarose gel stained with 20 µl of 1 mg/ml ethidium bromide was connected to a power supply at 80 V for 4 h. The loading dye

**Table 1.** Occurrence and distribution of enterobacteriaceae in commercial poultry feed types in Nigeria.

Feed category	<i>E. coli</i>	<i>S. paratyphi</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>	<i>Enterobacter</i> sp	<i>Yersinia</i> sp	<i>S. pullorum</i>	<i>S. enteritidis</i>	Others	*Overall % distribution by feed category
Chick mash	+	+	-	+	+	-	-	+	+	66.7 <sup>a</sup>
Growers mash	+	-	+	-	-	-	-	+	-	33.3 <sup>d</sup>
Layers mash	-	-	-	-	-	+	+	-	-	22.2 <sup>e</sup>
Broiler starter	+	+	+	-	+	-	-	-	+	55.6 <sup>b</sup>
Broiler finisher	+	-	+	+	+	-	-	-	-	44.4 <sup>c</sup>
% distribution	80	40	60	40	60	20	20	40	40	---
% occurrence ( <i>n</i> = 53)	28.3	3.8	34	3.8	15.1	1.9	1.9	5.6	5.6	---

*n* = total number of isolates; + = present; - = not present, \*Percentage values with different alphabets are significantly different at  $P < 0.05$

used was bromocresol purple. For each well, 15  $\mu$ l of plasmid DNA solution was mixed with 2  $\mu$ l loading dye, carefully loaded onto the gel and allowed to run for 2 h. DNA bands were visualized and photographed using Bio-Rad, Mini-Sub Gel GT<sup>®</sup>. The molecular weight of unknown plasmid DNA was extrapolated using the band mobilities in the gel. Cells from the primary bacterial colony (20  $\mu$ l) were cured in 0.1 mg/ml acridine orange supplemented nutrient broth as a confirmation of the presence of plasmid and its determinant in antibiotic resistance. Antibiotic susceptibility tests were repeated on the cured cells as confirmation of involvement of plasmid in antibiotic resistance.

#### Relationship between selected MDR strains from feed and morbid birds by plasmid analysis

The relationship between *E. coli* and *Salmonella* MDR strains from the feed samples and those from morbid birds showing signs of colibacillosis were determined. Ten morbid broiler birds from a poultry farm at Ijebu-ode, which have been fed with feed from the same batch that some of our feed samples were collected, showed the following morbidity signs: diarrhea, elevated blood protein levels, lack of appetite of food and loss of gross agility. Fecal samples stained with blood (indicating infection with hemolytic strains) were collected from each bird and subjected to microbiological analysis for *E. coli* and *Salmonella* following the methods of isolation and characterization stated previously. The antibiogram of the

45 isolates (data not shown) revealed 12 MDR *E. coli* and 7 MDR *Salmonella* strains which were then subjected to plasmid analysis as described above and comparison with plasmids of MDR strains from the feed samples by the similarity index. The similarity index ratio (S<sub>i</sub>) was defined as the relative proportion of the MDR strains of same species from one source that showed similar plasmid band size with MDR strains of same species from another source to the overall MDR strains of the species analyzed, all in percentage.

#### Statistical analysis

The overall percentage distributions of Enterobacteria in feed types were compared by the One-way ANOVA and statistically separated by the DMRT at  $P < 0.05$ . The Statgraphics version 5.0.1. (SAS Institute, NC, USA) was used for the analysis.

## RESULTS

### Incidence of enterobacteria in feed types

A total of 53 enterobacteria belonging to 5 genera (*Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter* and *Yersinia*) were isolated from the feed samples

(Table 1). *Klebsiella pneumoniae* was the most occurring species with 34% occurrence while *Yersinia* and *Salmonella pullorum* both had the least occurrence of 1.9%. Of the isolated *Salmonella*, *Salmonella enteritidis* occurred the most (37.5%) while *S. pullorum*, the least (12.5%) (data not shown). Table 1 also reveals that *E. coli* was the most distributed enterobacteria in feed types (80%). This pathogenic faecal indicator occurred in all feed types except layers mash samples while *S. pullorum* and *Yersinia* had 20% distribution and was recovered only from layers mash samples. Our data also show a significant ( $P < 0.05$ ) trend in the overall percentage distribution of contaminating enterobacteria across feed types with values of 66.7, 55.6, 44.4, 33.3 and 22.2% distribution in chick mash, broiler starter, broiler finisher, growers mash and layers mash, respectively.

### Response of enterobacteria to AST

From the reaction of the tested isolates to the different antimicrobials (Table 2), it is observed

**Table 2.** Percentage Antimicrobial Susceptibility Pattern for Isolates.

Antibiotics ( $\mu\text{g}$ )	<i>E. coli</i>			<i>K. pneumoniae</i>			<i>Yersinia sp</i>			<i>Enterobacter sp</i>			<i>S. typhi</i>			<i>S. enteritidis</i>		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Augmentin (30)	0	6.6	93.3	5.6	0	94.4	0	0	100	0	12.5	87.5	0	0	100	0	0	100
Ceftriaxone (30)	20	13.3	66.7	44.4	5.6	50	0	100	0	37.5	25	37.5	0	0	100	50	0	50
Nitrofurantoin (200)	0	0	100	5.6	11.1	83.3	0	0	100	0	0	100	0	0	100	0	50	50
Gentamycin (10)	53.3	6.7	40	44.4	22.2	33.3	0	0	100	50	12.5	37.5	50	0	50	100	0	0
Cotrimoxazole (25)	46.7	13.3	40	44.4	11.1	44.4	100	0	0	12.5	50	37.5	0	50	50	0	50	50
Ofloxacin (5)	60	13.3	26.7	61.1	5.6	33.3	100	0	0	50	12.5	37.5	50	50	0	100	0	0
Amoxicillin (25)	0	6.6	93.3	5.6	0	94.4	0	0	100	0	12.5	87.5	0	0	100	0	50	50
Ciprofloxacin (10)	66.7	26.7	6.7	61.1	0	38.9	100	0	0	37.5	12.5	50	100	0	0	100	0	0
Tetracycline (30)	26.7	33.3	40	50	16.7	33.3	0	100	0	12.5	50	37.5	50	50	0	100	0	0
Pefloxacin (5)	53.3	13.3	33.3	66.7	0	33.3	100	0	0	62.5	0	37.5	50	50	0	100	0	0

S – sensitive, I – intermediate, R – resistant.

that the isolates responded in varying patterns to the antimicrobial agents. The salmonellae and *Yersinia* showed higher sensitivity to the fluoroquinolones (ciprofloxacin, ofloxacin and pefloxacin), a trend which was not recorded for other isolates although all isolates were more sensitive to this class of antimicrobials. In terms of the overall response of the enterobacteria to tested antimicrobials (Figure 1), augmentin, nitrofurantoin and amoxicillin showed the least overall potency (<30%) against all isolates while ceftriaxone, cotrimoxazole and tetracycline showed an overall moderate potency (40 to 50%). However, the sensitivity to fluoroquinolones was above 70%. On the overall, 32.1% (17/53) of the isolates showed multidrug (MD) resistance. The MDR strains had a general drug resistant pattern of AUG-NIT-AMX. However, the COT pattern was additional for salmonellae while TET-CRO/COT for *E. coli* and *Enterobacter*.

### Plasmid profiles of MDR strains

The plasmid DNA profiles (Figure 2) showed that 71.4% (10/14) of the analyzed MDR strains possessed extrachromosomal determinants of MD resistance. The four tested *Klebsiella* isolates did not show any plasmid bands (data not shown). The relative sizes of the plasmid DNA obtained from the MDR strains ranged from 6.4 to 23.0 kb with the salmonellae having the lightest weight plasmids. The plasmid DNA size for *Enterobacter* was 11.3 kb.

### Similarity between selected MDR strains from feed and morbid birds

The MDR *E. coli* strains from the feed samples showed 78.9% similarity with those from the morbid birds as compared to the MDR strains of *Salmonella* from the feed samples which showed

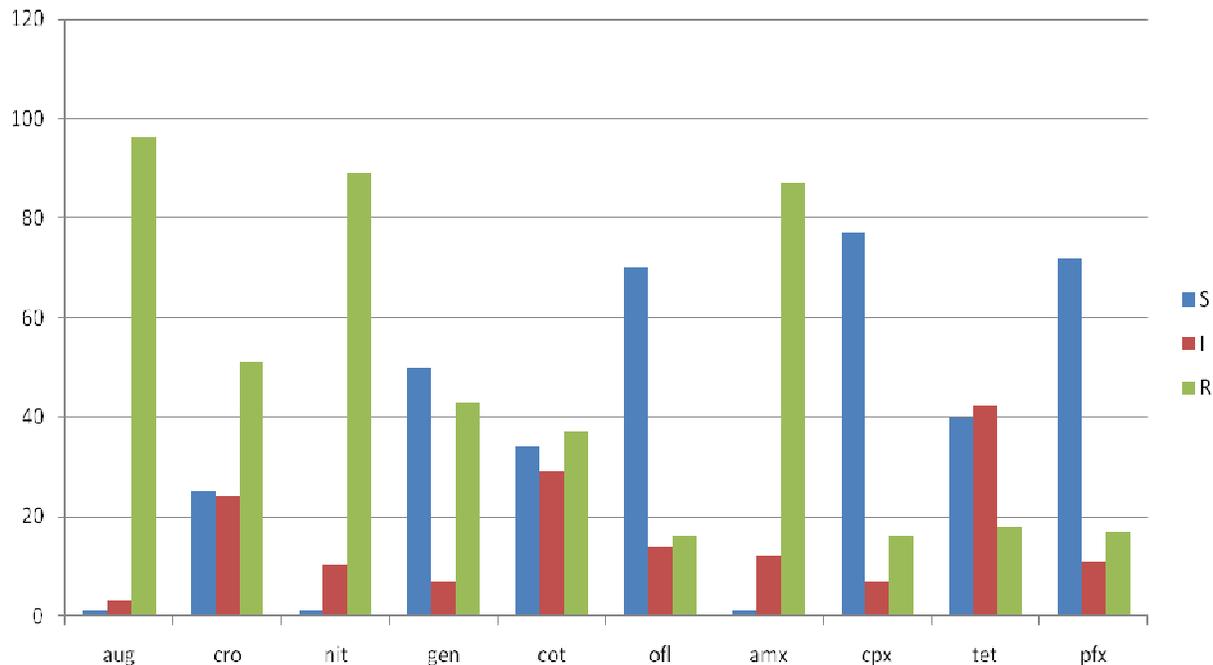
33.3% similarity with its MDR strains from the birds. Only one strain of MDR *S. enteritidis* (14.3%) was recovered from the morbid birds.

## DISCUSSION

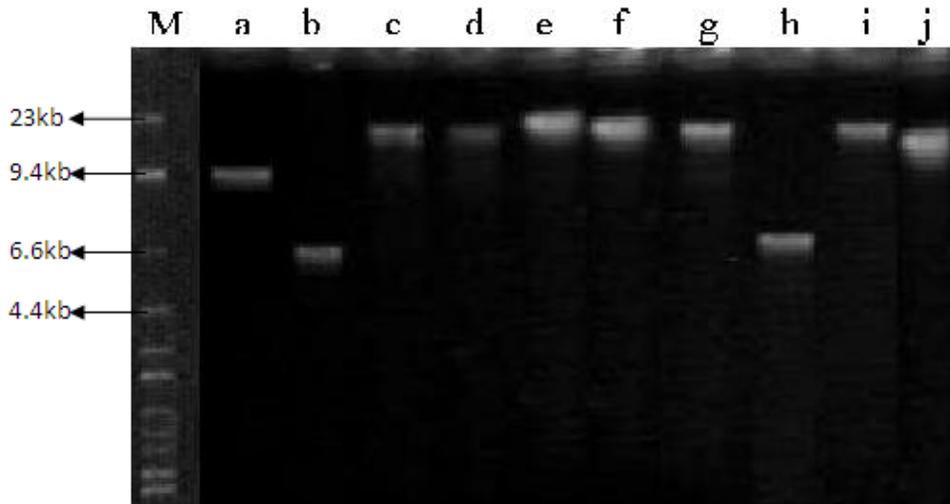
### Impact of incidence of enterobacteria in feed types on birds

The presence of Enterobacteriaceae in poultry feeds and consequently in the birds poses great economic and public health concern in terms of high mortality rate in birds due to colibacillosis and transfer of zoonotic agents like *Salmonella*, *Yersinia*, and *Escherichia*. Their presence indicates faecal contamination of raw materials, during processing and/or handling or packaging of finished product (Miranda et al., 2008).

The presence of the 5 genera of Enterobacteriaceae isolated in this study:



**Figure 1.** Overall percentage susceptibility pattern for the enteropathogens. S – sensitive, I – intermediate, R – resistant; aug – augmentin, cro – ceftriaxone, nit – nitrofurantoin, gen – gentamycin, cot – cotrimoxazole, ofl – ofloxacin, amx – amoxicillin, cpx – ciprofloxacin, tet – tetracycline, pfx – pefloxacin.



**Figure 2.** Plasmid bands of Multi Drug Resistant strains. M,  $\lambda$  DNA *Hind* III digested marker; a, *Enterobacter*; b, *S. typhi*; c-g and i-j, *E. coli*; h, *S. enteritidis*.

*Escherichia*, *Salmonella*, *Enterobacter*, *Yersinia*, and *Klebsiella*; is in agreement with the reports of Uwaezuoke et al. (2000) and Okoli et al. (2002). The occurrence of *S. pullorum* and *Salmonella paratyphi* in layer and starter feeds respectively, although at very low levels, raise serious safety questions bearing in mind that these two culprits have devastated the poultry industry directly by

inciting low egg production and quality in layers, and death in chicks (Barbour et al., 1983; Gast, 1997). Snoeyenbos (1991) suggested that the presence of *S. pullorum* in poultry feed is a potential hazard to the birds because when ingested could lead to possible vertical and horizontal transmission from infected parent to hatcher chicks and eggs. This may also be transferred to

the consumers of the birds. The presence of these bacteria in commercially available feeds in Nigeria therefore calls for attention in the production strategies employed by the poultry feed manufacturers. The role of poultry farmers in ensuring safety when handling animal droppings and contaminated feeds need not be under-emphasized. This is because most pathogenic bacteria contamination of feeds are either from handling during processing, packaging and/or storage, or the contaminated water sources used during pelleting of feeds (Cox and Pavic, 2010).

### Antibiogram reaction of isolates

The antimicrobials used against the isolates gave varying reactions. The best overall potency was seen with the fluoroquinolones (ofloxacin, ciprofloxacin and pefloxacin). However, the moderate resistant rates (37.5 to 50%) noticed with *Enterobacter* spp. against the fluoroquinolones are in accordance with the resistance rate observed by Uwaezuoke et al. (2000). In *E. coli*, the high values of resistance observed against augmentin (93.3%), ceftriaxone (66.7%), nitrofurantoin (100%) and amoxicillin (93.3%) in contrast to the appreciable sensitivity values (>50%) recorded towards the fluoroquinolones were in contradiction to the findings of Akond et al. (2009) who reported 82% resistance of *E. coli* from poultry and poultry environment in Bangladesh to fluoroquinolones, however, the report of Sharada et al. (2009) correlates with our data since they suggested a high sensitivity of *E. coli* from poultry in Bangalore to fluoroquinolones. The high resistance values in our study are similar to those observed in *E. coli* isolates from other sources against inexpensive, first-line broad-spectrum and readily available antibiotics (Chah et al., 2003; Okeke et al., 2000; Uwaezuoke et al., 2000).

*S. typhi* and *S. enteritidis* isolates of this study showed an overall resistance of 50 to 100% against augmentin, ceftriaxone, nitrofurantoin, cotrimoxazole and amoxicillin respectively but 85% susceptibility to fluoroquinolones. These values are similar with the report of Bower and Daeschel (1999) although the increased susceptibility of the salmonellae to fluoroquinolones contradicts the findings of Joshi and Amarnath (2007) who documented 100% resistance of salmonellae to fluoroquinolones (MIC >2 µg/l) but no MD resistance in *S. typhi*. Turner et al. (2006) also reported a full acquisition of fluoroquinolone resistance in *S. typhi* and suggested that such may be due to the accumulated point mutations in the topoisomerase targets. This may then imply that our salmonellae that were moderately sensitive to this class of antimicrobials may have some point mutations but yet to accumulate such so as to acquire full resistance. Therefore continuous exposure of these strains to low or high dosage of this antibiotic over time may change the tone and this will be detrimental to the poultry industry.

### Plasmid analysis for MDR strains and similarity between MDR strains

It is well known that MD resistance in microbes develop due to antibiotics misuse or acquisition of transferrable extra-chromosomal circular DNA called plasmids. Although we may not know the origin of the MD resistance exhibited by our MDR strains, we understand that the plasmids carry this special survival advantage since both low and high molecular weight plasmids were profiled from our strains, cured and mutants re-tested for antibiotic susceptibility. The sizes of the bands from *S. typhi* (6.4 kb) and *S. enteritidis* (6.7 kb) suggest that these plasmids encoding MD resistance in the salmonellae could be mobilizable plasmids rather than conjugative plasmids since mobilizable plasmids are usually less than 10 kb in size while conjugative ones are sized between 20 and 30 kb (Esimone et al., 2010). Conversely the heavy weight plasmids detected in MDR strains of *E. coli* and *Enterobacter* maybe conjugative, more so when these strains were all determined to be motile. Therefore their resistance determinants may have been acquired by horizontal transfer from species. The curing of primary inoculum further validated the fact that plasmids were responsible for MD resistance as all cured cells lost their plasmids and showed 100% sensitivity to all previously resistant antibiotics except augmentin and nitrofurantoin. This may then indicate that genes encoding resistance to these two antibiotics are chromosomal in our MDR strains. The absence of plasmids in our MDR *Klebsiella* may also indicate chromosomal control other than plasmid. The fact that these resistant strains have the capacity to colonize, though transiently, the human gut and/or exchange their R-factor (Resistance transfer factor) with the intestinal flora of humans thereby increasing the spread of MD resistance makes our findings more complicating.

Regarding the similarity of MDR strains from feed samples and morbid birds which may be used as an index for pathological studies, we recorded some certain high level of similarity among the *E. coli* MDR strains. It is possible that the birds acquired these virulent MDR strains from feed consumption since the  $S_i$  was quite high (78.9%). Nijsten et al. (1994) and (1996) recorded significantly higher resistant *E. coli* in the fecal Enterobacteriaceae of pig farmers than in pig slaughterers and urban residents while Van den Bogaard and Stobberingh (2000) and Van den Bogaard et al. (2001) suggested a possible transfer of R plasmids across species (animals to man) and vice versa. Although we did not work on the relationship of MDR strains in feed and morbid birds to those occurring in the breeders, we can infer from the data we have so far and those of previous studies, that there may have been the transfer of R plasmids across species (animals to man) with evidence of transfer within species. However, the lower  $S_i$  (33.3%) recorded for the *Salmonella* MDR

strains show virtually no significant relationship between MDR strains from feed and those occurring in morbid birds. It is possible that the MDR salmonellae isolated from the morbid birds were acquired via other sources.

In the light of the data reported herein we conclude that poultry feeds available in Nigeria are unsafe for consumption by the birds due to the high occurrence rates and wide distribution of enteropathogens including MDR strains. There is also an indication of direct transfer of pathogenic MDR strains of enterobacteria from feed to birds. Following our reports and previous findings of Bates et al. (1994), Blanco et al. (1997) and Garau et al. (1999), it can further be argued that the load of as much as 60% of annually produced antibiotics which are added to livestock feed in Nigeria, though at prophylactic levels, may serve as a contributing factor to increasing drug resistance in both animal and human bacterial isolates. In a manuscript under review, Ezekiel et al. reported the presence of trace levels (as low as 3 µg/kg) of chloramphenicol and other antibiotics in the LC-MS/MS analysis of our feed samples for 63 fungal and bacterial metabolites. This further suggests that in addition to the incorporation of antibiotics in feed formulation, some contaminating bacteria such as *Streptomyces* secrete these metabolic by-products in order to out-compete other contaminants within the feed. However, MDR strains may adapt and survive within the feed by acquiring the extrachromosomal determinants. Therefore not only that the birds are at risks, poultry workers and consumers are equally exposed to serious hazards due to MD resistance. This calls for urgent intervention by regulatory agencies so that aseptic measures from start to finish in the feed production process can be effected (Noordhuizen and Frankena, 1994).

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## REFERENCES

- Aarestrup FM (2000). Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. *APMIS*, 108(Suppl. 101): 5–48.
- Akond MA, Hassan SMR, Alam S, Shirin M (2009). Antibiotic resistance of *Escherichia coli* isolates from poultry and poultry environment of Bangladesh. *Am. J. Environ. Sci.*, 5(1): 47–52.
- Barbour EK, Nabbut NH, Hinners SW (1983). Distribution of paratyphoids on Saudi Arabian poultry farms and pathogenicity studies of predominant serotypes. *Avian Dis.*, 27(3): 616–622.
- Bates J, Jordens JZ, Griffiths DT (1994). Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. *J. Antimicrob. Chemother.*, 34: 507–516.
- Bhaduri S, Cottrell B, Pickard AR (1997). Use of a single procedure for selective enrichment, isolation, and identification of plasmid bearing virulent *Yersinia enterocolitica* of various serotypes from pork samples. *Appl. Environ. Microbiol.*, 63(5): 1657–1660.
- Blanco JE, Blanco M, Mora A, Blanco J (1997). Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *J. Clin. Microbiol.*, 35: 2184–2185.
- Bower CK, Daeschel MA (1999). Resistance responses of microorganisms in food environments. *Int. J. Food Microbiol.*, 50: 33–44.
- Chah KF, Okafor SC, Oboegbulem SI (2003). Anti-microbial resistance of none clinical *E. coli* strains from chicken in Nsukka, Southeastern Nigeria. *Nig. J. Anim. Prod.*, 30: 101–106.
- Cowan SR, Stell KJ (1993). *Manual for Identification of Medical Bacteria*, pp. 140–143. 3<sup>rd</sup> Ed. Cambridge University Press, Uk.
- Cox JM, Pavic A (2010). Advances in enteropathogen control in poultry production. *J. Appl. Microbiol.*, 108: 745–755.
- Da Costa PM, Oliveira M, Bica A, Vaz-Pires P, Bernardo F (2007). Antimicrobial resistance in *Enterococcus spp.* and *Escherichia coli* isolated from poultry feed and feed ingredients. *Vet. Microbiol.*, 120: 122–131.
- Esimone CO, Nworu CS, Harrison GT (2010). Antibigram and plasmid profile of some multi-antibiotics resistant urinopathogens obtained from local communities in Southeastern Nigeria. *Ibinosina J. Med. BS.*, 2(4): 152–159.
- Garau J, Xercavins M, Rodríguez-Carballeira M, Gómez-Vera JR, Coll I, Vidal D, Llovet T, Ruíz-Bremón A (1999). Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. *Antimicrob. Agents Chemother.*, 43: 2736–2741.
- Gast RK (1997). Detecting infections of chickens with recent *Salmonella pullorum* isolates using standard serological methods. *Poult. Sci.*, 76(1): 17–23.
- ISO Standard 7402 (1993). General guidance for the enumeration of enterobacteriaceae without resuscitation – MPN technique and colony count technique. Geneva, Switzerland: International Organisation for Standardisation.
- Joshi S, Amarnath SK (2007). Fluoroquinolone resistance in *Salmonella typhi* and *S. paratyphi A* in Bangalore, India. *Trans. R. Soc. Trop. Med. Hyg.*, 101(3): 308–310.
- Kraft R, Tardiff J, Krauter KS, Leinwand LA (1988). Using mini-prep plasmid DNA for sequencing double stranded template with sequences. *Bio. Tech.*, 6: 544.
- Lech K, Brent R (1987). Mini-preps of plasmid DNA. P. 1.6.1 – 1.6.4. In Ausubel et al. (eds.), *Current protocols in Molecular Biology*. John Wiley and Sons, NY.
- Micro livestock (1991). A little known small animals with a promising economic future, pp. 79–160. National Academic Press, Washington.
- Miranda JM, Guarddon M, Vazquez BI, Fente CA, Barros-Velazquez J, Cepeda A, Franco CM (2008). Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey. *Food Contr.*, 19: 412–416.
- National Committee for Clinical Laboratory Standards (2002). Performance standards for antimicrobial susceptibility testing, 12<sup>th</sup> informational supplement. M100-S12, NCCLS, Wayne, PA.
- Nijsten R, London N, van den Bogaard A (1994). Resistance in faecal *Escherichia coli* isolated from pig farmers and abattoir workers. *Epidemiol. Infect.*, 113: 45–52.
- Nijsten R, London N, van den Bogaard A (1996). Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmers and pigs. *J. Antimicrob. Chemother.*, 37: 1131–1140.
- Noordhuizen JP, Frankena K (1994). *Salmonella enteritidis*: clinical epidemiological approaches for prevention and control of *S. enteritidis* in poultry production. *Int. J. Food Microbiol.*, 21(1–2): 131–143.
- Obi CN, Ozugbo IJ (2007). Microbiological Analyses of Poultry Feeds Sold in Umuahia Main Market, Abia State, Nigeria. *Res. J. Appl. Sci.*, 2(1): 22–25.
- Okeke IN, Fayinka ST, Lamikanra A (2000). Antibiotic Resistance in *E. coli* from Nigerian Students, 1986–1998. *Emerg. Infect. Dis.*, 6: 393–396.
- Okoli IC, Ozoh PTE, Udedibie ABI (2002). Epizootiological and microbiological methodologies for monitoring anti-microbial resistance among Enterobacteriaceae of animal origin. A review. *Nig.*

- Vet. J., 23: 23–39.
- Paterson DL (2006). Resistance in Gram-negative bacteria: Enterobacteriaceae. *Am. J. Med.*, 119: 520–528.
- Saenz Y, Zarazaga M, Brinas L, Lantero M, Ruiz-Larrea F, Torres C (2001). Antibiotic resistance in *E. coli* isolates obtained from animals, foods and humans in Spain. *Intl. J. Antimicrob. Agents*, 18: 353–358.
- Sharada R, Ruban SW, Thiyageeswaran M (2009). Antibiotic resistance pattern of *Escherichia coli* isolated from poultry in Bangalore. *Intl. J. Microbiol.*, 7(1): e31.
- Smith WT (2005). Feed Chickens Properly Co-operative Extension, Extension Service of Mississippi State University Service., pp. 58–60.
- Snoeyenbos GH (1991). Pullorum disease, pp. 73–86. In B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder Jr., (eds.), *Diseases of poultry*, 9<sup>th</sup> Ed. Iowa State University Press, Ames.
- Talaro KP, Talaro A (2002). *Foundations in Microbiology*, 4<sup>th</sup> Ed. The McGraw Hill Companies, NY, pp. 398–400.
- Turner AK, Nair S, Wain J (2006). The acquisition of full fluoroquinolone resistance in *Salmonella typhi* by accumulation of point mutation in the topoisomerase targets. *J. Antimicrob. Chemother.*, 58: 733–740.
- Uwaezuoke JC, Ogbulie JN, Njoku JN, Obiajuru IOC, Njoku AJ (2000). Antibiotic sensitivity patterns of bacterial isolates from poultry feed. *Intl. J. Environ. Health Hum. Dev.*, 1: 23–28.
- Van den Bogaard AE, London N, Driessen C, Stobberingh EE (2001). Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *J. Antimicrob. Chemother.*, 47: 763–771.
- Van den Bogaard AE, Stobberingh EE (2000). Epidemiology of resistance to antibiotics. Links between animals and humans. *Intl. J. Antimicrob. Agents*, 14: 327–335.