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Research Article
Molecular Studies of Antibiotics Resistant Bacteria Contaminating Poultry Feeds in Enugu Metropolis, Nigeria: The Public Health Implications

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Abstract
Background and Objectives: Poultry refers to all domesticated birds kept for egg laying or meat production. Poultry feeds are feedstuffs used in raising poultry birds. Unsafe feeds may cause chronic losses to farmers and also pose public health risk. This study was conducted to identify and characterize bacterial flora of poultry feeds. **Materials and Methods:** Locally milled and company branded poultry feeds were obtained within Enugu, Nigeria. A total of three hundred samples of poultry feeds were investigated for bacterial contamination and total bacterial counts. Susceptibility pattern of two bacterial isolates to commonly used antibiotics was determined *in vitro* employing standard disk diffusion method. Molecular studies of four bacterial isolates with multiple drug resistance were done using Agarose Gel electrophoresis for plasmid profiling, multiplex PCR was used to detect SHV and CTX-M gene while conventional linear PCR was used to detect TEM gene. **Results:** A total of six bacterial species were isolated and they were *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus* species and *Yersinia* specie. The total bacteria counts ranged from 1.92×10^{4}–25.0×10^{6} CFU g^{-1}. *Escherichia coli* having the highest occurrence rate (45.7%) was highly resistant to tetracycline (92%), streptomycine (90%), neomycin (90%), ampicillin (80%) and chloramphenicol (78%). Molecular studies of the isolates showed presence of one plasmid DNA each with band weight of 17-18 kb. *Escherichia coli* and *Klebsiella pneumonia* were positive for SHV and TEM gene while only *Escherichia coli* were positive for CTX-M. **Conclusion:** Poultry feeds did not meet the international microbiological standards.

Key words: Antibiogram profiles, bacterial isolates, environmental contamination, microbial contamination, poultry feed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The term 'poultry' refers to all domesticated birds kept for egg laying or meat production. Poultry farming, one of the most important divisions of agriculture throughout the world is a source of palatable, economical and healthy food protein[1]. Poultry is the second most widely eaten meat in the world, accounting for about 30% of the world meat[2]. The rapid growth in the meat sector has been underpinned by a rising demand for poultry meat, which has increased at around three times the rate of population growth over each of the past five decades[3].

The effective development of poultry birds depends on the quality of water, breeds, environmental conditions, disease control, equipment and farming practices of which poultry feed is the most important and critical factor[4]. Poultry feeds are food materials used in raising birds and are designed to contain all the nutritional materials needed for proper growth, meat and egg production.

Poultry feeds composed largely of grains such as corn, wheat or barley, oil seeds, peanuts and protein products of animal origin such as fish meal, meat and bone meal[5]. Poultry feeds can be classified into various types based on what they are intended to accomplish in poultry, for example starter, grower, finisher, layer and broiler category. As poultry are regularly exposed to contamination from diverse sources, poultry feed is one of the most important sources of contamination including environmental pollution, microbes and activities of insects[6]. The use of poor quality ingredients has led to the production of poor quality feeds. Public health depends on the quality of poultry feeds because it affects the wholesomeness of poultry meat consumed by man.

Specifically, some additives have been implicated as major sources of bacteria in poultry feeds[7]. Poultry diseases may be caused by pathogenic organisms, nutritional deficiency and cannibalism. Some of poultry diseases are Newcastle disease, fowl typhoid, fowl pox disease and chronic respiratory disease. Bacteria organisms that can contaminate poultry feeds include *Escherichia coli*, *Salmonella* specie, *Shigella* specie, *Staphylococcus aureus* and *Klebsiella* specie. Bacterial contamination of poultry feeds re-enforces the need for proper hygiene in the processing, packaging, storage and transport of poultry feeds.

In modern poultry production, antibiotics are used for treatment and prevention of infection and diseases. These antibiotic treatments are considered to promote the emergence, selection and spread of antibiotic resistant micro-organism in both veterinary and human medicine. As such, the transmission of plasmid mediated resistance between different bacterial species have now widely occurred[8]. Consumption of poultry birds containing multi-drug-resistant isolates has raised concern about the horizontal transfer of these resistant genes to human. Hence, a ban has been placed on use of antibiotics in poultry production in Europe, United States and Australia[9]. Production of poultry feeds requires adequate microbiological safety regulations. Lateef *et al.*[10] has observed that the production of poultry feeds for local and commercial farmers in the developing countries including Nigeria requires adequate microbiological safety regulations to escape microbial contamination of the product. Therefore, this study was designed to evaluate the molecular characterization of antibiotics resistant bacteria contaminating poultry feeds.

MATERIALS AND METHODS

Collection of poultry feeds samples: A total of 120 samples of locally milled poultry feeds and 180 samples of mechanized company compounded poultry feeds were collected into a sterile polyethylene bags and taken to the laboratory, properly ground to fine particles (Imm particle size) using the mill attachment of a Moulinex Blender before analysis.

Total bacterial counts of the samples: Only 1 g of each poultry feed was homogenized in 9 mL of sterile water in a test tubes. Serial dilution was carried out to 10^{-12} dilution. About 0.1 mL aliquot of 10^{-10}, 10^{-11} and 10^{-12} diluted sample was inoculated in triplicate by the spread plate technique on Nutrient agar plates. The plates were incubated at 37°C for 24 hrs after which the colonies were counted.

Isolation of the bacterial isolates: Only 1 g of each poultry feed was homogenized in 9 mL of sterile distilled water in a test tube. Serial dilution was carried out to 10^{-5} dilution. About 0.5 mL aliquot of 10^{-5} diluted sample was inoculated onto already prepared and solidified Nutrient agar, MacConkey agar and Eosin Methylene Blue agar. The plates were incubated at 37°C for 24 hrs and the colonies were observed and picked individually from the initial culture and streaked onto already prepared Nutrient agar plates. The isolation of pure culture was performed by continuous sub-culturing until pure culture was obtained.

Characterization and identification of bacterial isolates: Pure cultures of bacterial isolates were characterized and identified using various biochemical tests as established by Holt[11].

Antibiotic susceptibility testing: Susceptibility pattern of the bacterial isolates to commonly used antibiotics was determined *in vitro* by employing standard disk diffusion method.

Determination of number and size of bacterial plasmids: Molecular studies of bacterial isolates with very high multi-drug resistance were carried out. The pure cultures of these selected isolates were stored in a refrigerator of 4-8°C. The bacterial isolates were subjected to plasmid DNA isolation following the protocol of Caratolli[12]. The bacterial culture was centrifuged at 6,000 rpm for 5 min at 4°C. The supernatant was decanted and 1 mL of sterile distilled water or PBS was added to pellet and the cells were re-suspended. These cells were centrifuged at 6000 rpm for 5 min. The supernatant was removed leaving only about 100 µL with the cells. Thereafter, 300 µL of lysis solution was added and mixed thoroughly by inverting the tube 4-6 times until solution was viscous and slightly clear.

It was incubated at room temperature for 5 min. (The SDS in the lysis solution dissolves the lipid component of the cell. The high pH of the solution also denatures the chromosomal and plasmid DNA). Thereafter, 150 µL of 3.M sodium acetate solution was added, vortexed to mix and incubated at room temperature for 5-10 min. The sodium acetate returned the pH to neutral allowing the DNA strands to renature. The cells were spinned at 12 k rpm (20,000 X g) for 10 min at 4°C to pellet cells debris and chromosomal DNA. The supernatant was transferred to another eppendorf tube and 900 µL of ice cold absolute ethanol was added to it. It was mixed well to precipitate out the plasmid DNA. It was then spinned again at high speed in an eppendorf centrifuge for 10 min to pellet the DNA. The supernatant was aspirated away from the DNA pellet and discarded. Afterward, 1ml of cold 70% ethanol was added to the DNA pellet which was washed by inverting the tube several times and spinned at high speed for 10 min. The supernatant was aspirated away from the DNA pellet and discarded. The DNA pellet was air dried for at least 10 minutes. The dried DNA pellet was re-suspended in 20-40 µL of TE buffer or distilled water for further use. Afterward, 7 µL of plasmid DNA were mixed with 3 µL of DNA loading buffer dye and loaded into 1% Agarose gel stained with ethidium bromide (1 µg mL^{-1}). A 1kb DNA ladder was used as DNA molecular weight marker. Electrophoresis was carried out at 90 volts until the sample dye front are near the end of the gel. The agarose gel was visualized under ultraviolet transillumination.

Molecular characterization of the bacterial isolates: Multiplex PCR was used to detect SHV and CTX-M genes as described by Poirel *et al.*[13] while conventional linear PCR was done for Bla TEM type ESBL gene.

RESULTS

A total of six bacterial species were isolated from the poultry feeds. The isolated bacteria were tentatively identified as *Escherichia coli*, *Klebsiella pneumonia*, *Bacilluscereus*, *Staphylococcus aureus*, *Enterococcus* species and *Yersinia* specie. These organisms were distributed throughout all the feeds samples (Table 1 and 2). The bacterial loads of the poultry feeds were presented in Table 3. The bacterial loads were in the range of $1.92\times10\ 10^4$-25.0×10^6 CFU g^{-1} with brand CM (starter feed) having the highest number of bacterial contamination while brand VM (grower feed) had the least bacterial load. The bacteria isolated from the poultry feeds showed varying degree of resistance while a few drugs were highly sensitive against the bacterial isolates (Table 4 and 5). *Escherichia coli* showed highest resistance against tetracycline (92%) followed by streptomycine (90%), Neomycine (90%), ampicillin (80%), chloramphenicol (78%) and showed low resistance to ofloxacine (6%), ciprofloxacin (6%), ceftriaxone (5%) and amoxil (4%). *Klebsiella pneumonia* showed the highest resistance against ampicillin (95%) followed by tetracyclin (92%), Neomycin (91%), Streptomycin (90%), Chloramphenicol (80%) and no resistance (highly sensitive) against ciprofloxacin, afloxacin, ceftriaxone and amoxil. *Klebsiella pneumonia* was completely susceptible to ciprofloxacin, ofloxacin, ceftriaxone and amoxil. The molecular

Table 1: Percentage occurrence of bacteria isolated from poultry feeds in Enugu metropolis

Bacteria	No isolate
Escherichia coli	153 (45.7%)
Bacills cereus	101 (30.1%)
Straphylo coccus aureus	8 (2.4%)
Klebsiella pneumonia	30 (9%)
Yersinia specie	2 (0.6%)
Enterococcus specie	41 (12.2%)
Total	335 (100)

Table 2: General distribution of bacterial isolates in locally milled and company branded feeds

Bacteria	LM	MCC
Escherichia coli	58	95
Bacills cereus	37	64
Straphylococcus aureus	7	0
Klebsiella pneumonia	8	22
Yersinia specie	14	27
Enterococcus specie	0	2

Table 3: Mean bacterial count of poultry feed samples in Enugu metropolis

TM starter	TM grower	TM finisher	VM starter	VM grower	VM finisher	HM starter	HM grower	HM finisher
4.4×10^5	5.6×10^5	12.9×10^5	1.92×10^4	2.1×10^4	3.4×10^4	7.4×10^4	5.2×10^4	2.7×10^4
CM layer	CM finisher	CM starter	Spent grain	Palm kernel cake	Bone meal	Soya fat	TM layer	Blood meal
10×10^5	18.8×10^5	25×10^6	16.8×10^4	6.3×10^4	13.1×0^4	3.0×10^4	8.9×10^5	Numerous

Table 4: Antimicrobial susceptibility pattern of *Escherichia coli* isolated from the poultry feeds in Enugu metropolis

Antibiotics	Resistivity (%)	Sensitivity (%)
Tetracycline	92	8
Streptomycine	90	10
Neomycine	90	10
Ampicillin	80	20
Chloramphenicol	78	22
Ciprofloxacine	6	94
Ofloxacin	6	94
Ceftriaxone	5	95
Amoxil	4	96

Table 5: Antimicrobial susceptibility pattern of *Klebsiella pneumonia* isolated from the poultry feeds in Enugu metropolis

Antibiotics	Resistivity (%)	Sensitivity (%)
Tetracycline	92	8
Streptomycine	90	10
Neomycine	91	9
Ampicillin	95	5
Chloramphenicol	80	20
Ciprofloxacine	0	100
Ofloxacin	0	100
Ceftriaxone	0	100
Amoxil	0	100

Table 6: Number and sizes of bacterial plasmids isolated from poultry feeds in Enugu Metropolis

Isolates	No. of plasmids	Molecular weight (kb)
Escherichia coli	1	18
Klebsiella pneumonia	1	18
Bacillus cereus	1	18
Staphylococcus aureus	1	17

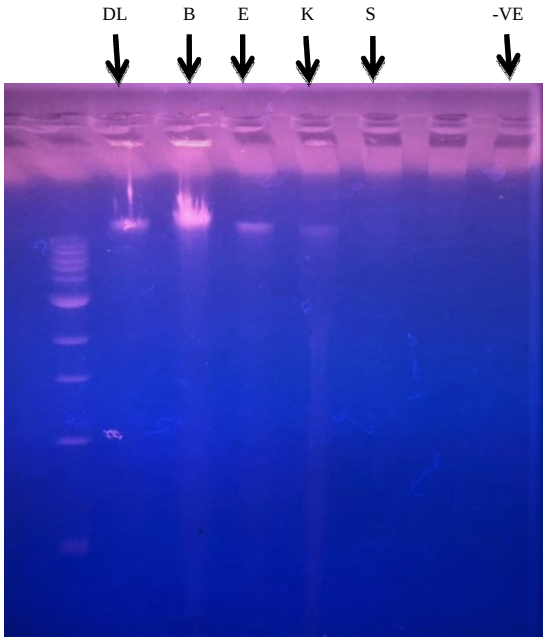

Fig. 1: Plasmid profile of bacterial isolates

B: Supreme Grower (*Bacillus cereus*), E: Palm Kernel Cake (*Escherichia coli*), K: Hybrid Layer (*Klebsiella pneumoniae*), S: Bone Barrow (*Staphylococcus aureus*), DL: DNA Ladder and -ve: Negative control
All the isolates have plasmid DNAs of approximately the same molecular weight (18 kb)
Summary of result: All the isolates have plasmid DNAs of approximately the same molecular weight (18kb)

studies of the isolates with high multi-drug resistance were presented in Table 6 and Fig. 1. The results showed the number of plasmids and their sizes. The four bacterial isolates studied had a single plasmid DNA each. The molecular weights of these plasmid DNAs ranged from 17-18 kb.

Molecular characterization of the two selected bacterial isolates (*Escherichia coli* and *Klebsiella pneumonia*) were recorded in Fig. 2 and 3. *Escherichia coli* and *Klebsiella pneumonia* were positive for SHV gene and TEM gene while only *Escherichia coil* was positive for CTV-M gene.

DISCUSSION

All the poultry feeds investigated in this study showed the presence of bacterial contaminants which were *Escherichia coli* 153(45.7%), *Bacillus cereus* 101(30.1%), *Enterococcus* specie 41(12.2%), *Klebsiella pneumonia* 30(9%), *Staphylococcus aureus* 8(2.4%) and *Yersinia* specie 2(0.6%). *Escherichia coli* were found to be the most widely distributed isolates while Yersinia specie were the least distributed. The high incidence of *Escherichia coli* is an indication of faecal contamination of the feeds associated with poor hygiene[14]. Some of the bacterial isolates belonged to the *Enterobacteriaceae* family, which can cause gastro-intestinal tract infections in poultry birds and human handlers[15]. Gyang *et al.*[16] had isolated *Escherichia coli* (25%), S*taphylococcus aureus (*14%), *Bacillus cereus* (34%), *Klebsiella* specie (6%) and *Enterococcus* specie (12%) which agrees with the findings of the present study. These micro-organisms may came from the raw materials used to make the feeds. However, most bacterial micro-organisms originate from air and soils[17]. Results of the present study agrees with the findings of Dhand *et al.*[6] and Hancock *et al.*[18] who separately implicated *Bacillus cereus* and *Staphylococcus aureus* in the microbial infection outbreak of poultry farming. In the present study, *Staphylococcus aureus* was found in poultry feeds,

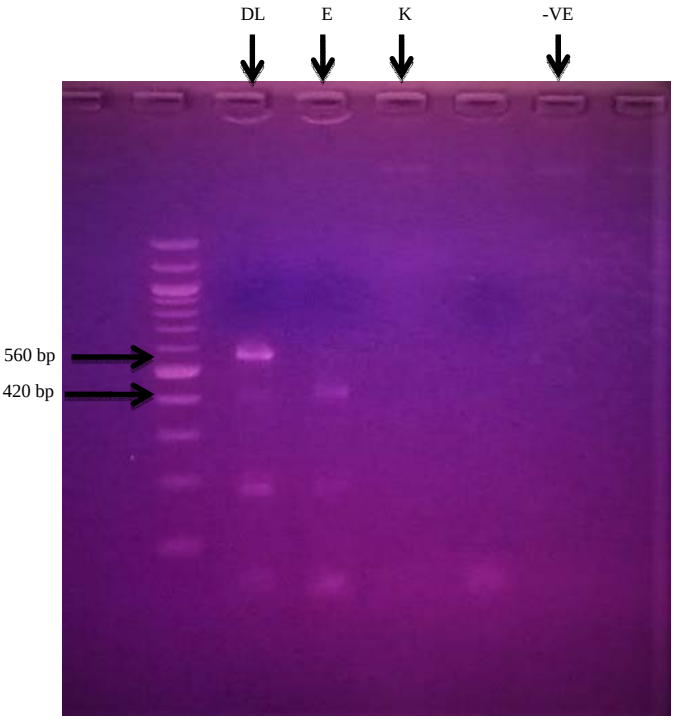

Fig. 2: CTX-M gene (560 bp) and SHV gene (420 bp) gel image

E: Palm Kernel Cake (*Escherichia coli*), K: Hybrid layer (*Klebsiella pneumoniae*), DL: DNA ladder and -ve: Negative control, Isolates E and K were positive for the SHV gene, while only isolate E was positive of the CTX-M type ESBL gene

Summary of result: Isolates E and K were positive for the SHV gene, while only isolate E was positive of the CTX-M type ESBL gene

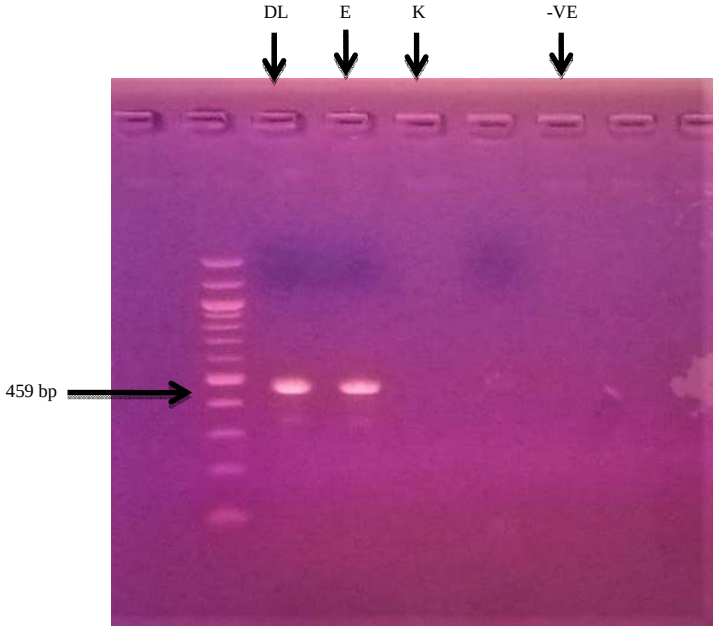

Fig. 3: TEM gene (459 bp) gel image

E: Palm kernel cake (*Escherichia coli*), K: Hybrid layer (*Klebsiella pneumonia*), DL: DNA ladder and -ve: Negative control, Isolates E and K were both positive for the TEM gene

Summary of result: Isolates E and K were both positive for the TEM gene

suggesting contamination by local millers or market sellers. There is a potential health risk for poultry birds, human handlers and consumers of poultry meat due to the high incidence of bacterial species in poultry feeds. The bacterial loads of the poultry feeds were in the range of 1.92×10^4-25.0×10^6. If the animal feeds exceeds 300,000 CFU g^{-1} for older animals and 500,000 CFU g^{-1} for younger animals, they are regarded as non-compliant with international microbiological standards[19]. The microbiological quality of all the feeds in this study was poor. High numbers of bacterial isolates in feeds suggest that the feeds contain sufficient nutrients for bacterial growth and by causing degradation, these organisms reduce the available nutrients for birds. According to the antimicrobial susceptibility test, *Escherichia coli* isolates are highly resistant to tetracycline (92%), streptomycin (90%), neomycin (90%), ampicillin, (80%), chloramphenicol (78%) and strongly sensitive to ciprofloxacine (94%), afloxacin (94%), ceftriaxone (95%) and amoxil (96%). The antimicrobial susceptibility test also showed that the *Klebsiella pneumonia* isolate is strongly resistant to ampicillin (95%), tetracycline (92%), neomycine (91%), streptomycin (90%), chloramphenicol (80%) and strongly sensitive to ciprofloxacin (100%), afloxacin (100%), amoxil (100%) and ceftriaxone (100%). The susceptibility pattern observed in the present study are comparable to those reported by Onyeze *et al.*[20] and Gyang *et al.*[16]. Some of these bacterial isolates have evolved into multi-drug resistant forms because of the use of antibiotics in poultry farming. There should be concern over the antibiotic susceptibility pattern obtained from this recent study, since in some parts of the world, a ban had been imposed on the use of antibiotics for disease prevention and control in poultry farming. WHO reported that only tetracyclines are allowed for use in poultry disease prevention and as growth promoters. This is worrisome because Several bacterial isolates from the poultry feeds in this recent study showed strong resistance to tetracycline (92%) indicating that these isolates over time have acquired resistant genes that may be horizontally transferred from one species to another. The molecular studies of these bacterial isolates with high multi-drug resistance showed that the four bacterial isolates studied had one plasmid DNA each with varying molecular weight of 17-18 kb. This result agrees with the findings of Gyang *et al.*[16] who reported that *Escherichia coli*, *Salmonella* specie, *Staphylococcs aureus* and *Klebsiella pneumoniae* had one plasmid DNA each. The molecular weights of the bacterial isolates noted in the current study ranged from 17-18 kb suggesting that these plasmids could be conjugative since mobilised plasmids are usually less than 10 kb[21]. The conjugative plasmids may have been acquired by horizontal transfer from one species to another.

Escherichia coli and *Klebsiella pneumoniae* were positive for TEM gene and SHV gene while only *Escherichia coli* was positive for CTX-M gene. This indicates that poultry feed is a potential source of these multi-drug resistant bacterial isolates which were responsible for serious human and animal health concern worldwide.

CONCLUSION

Poultry feeds analysed in this study contained high levels of bacteria and most of these isolates were common environmental contaminants. All feeds investigated failed to meet international microbiological standards. Poultry feed manufacturers should be encouraged to establish a quality control algorithm to be adopted during feed formulation, storage, usage, transport and retailing.

REFERENCES

1. Mahesar, S.A., S.T.H. Sherazi, A. Niaz, M.I. Bhanger and A. Rauf, 2010. Simultaneous assessment of zinc, cadmium, lead and copper in poultry feeds by differential pulse anodic stripping voltammetry. Food Chem. Toxicol., 48: 2357-2360.
2. Ellis, E.M., 1969. Salmonella reservoirs in animals and feeds. J. Am. Oil Chem. Soc., 46: 227-229.
3. FAO, 2013. FAO Statistical Yearbook 2013: World Food and Agriculture. Food and Agriculture Organization, Rome, Italy, ISBN-13: 9789251073964, Pages: 356.
4. Nazri, M.M., 2003. Development of Poultry Industry. Economic Review, March 2003 Issue.
5. Bale, O. O. J., A.A. Sekoni and C.N. Kwanashie, 2002. A case study of possible health hazards associated with poultry houses. Nigerian J. Anim. Prod., 29: 1022-1031.
6. Dhand, N.K., D.V. Joshi and S.K. Jand, 1998. Contamination of dairy feeds and their toxigenicity. Indian J. Anim. Sci., 68: 1095-1096.
7. Ogbulie, J.N. and G.C. Okpokwasili, 1999. Haematological and histological responses of *Clarias gariepinus* and *Heterobranchus bidorsalis* to some bacterial diseases in River State, Nigeria J. Nat. Sci. Found. Sri Lanka, 27: 1-16.
8. Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science, 264: 375-382.
9. Ezekiel, C.N., A.O. Olarinmoye, J.M.A. Oyinloye, O.B. Olaoye and A.O. Edun, 2011. Distribution, antibiogram and multidrug resistance in *Enterobacteriaceae* from commercial poultry feeds in Nigeria. Afr. J. Microbiol. Res., 5: 294-301.

10. Lateef, A. and E.B Gueguim-Kana, 2014. Quality assessment and hazard analysis in the small-scale production of poultry feeds in Ogbomoso, Southwest Nigeria Qual. Assur. Saf. Crops Foods, 6: 105-113.
11. Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams and Wilkins, Baltimore, USA., ISBN-13: 9780683006032, Pages: 787.
12. Carattoli, A., 2003. Plasmid-mediated antimicrobial resistance in *Salmonella enterica*. Curr. Issues Mol. Biol., 5: 113-122.
13. Poirel, L., T. Naas, I.L. Thomas, A. Karim, E. Bingen and P. Nordmann, 2001. CTX-M-type extended-spectrum β-lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. Antimicrob Agents Chemother, 45: 3355-3361.
14. Matthew, O., 2017. Microbial analysis of poultry feeds produced in Songhai farms, Rivers State, Nigeria. J. Microbiol. Exp., Vol. 4 10.15406/jmen.2017.04.00110.
15. WHO., 2011. European strategic action plan on antibiotic resistance. World Health Organization Regional Office for Europe. https://www.euro.who.int/__data/assets/pdf_file/0008/147734/wd14E_AntibioticResistance_111380.pdf.
16. Gyang, L., S.O. Obiekezie, J.E. Owuna, M.O. Adamu and S.O. Obiekezie, 2019. Bacterial contamination of poultry feeds, molecular studies and antibacterial resistance profiles of isolates in Keffi metropolis, Nigeria. Int. J. Eng. Sci., 8: 14-19.
17. Arotupin, D.J. and F.A. Akinyosoye, 2001. Evaluation of microbial isolates from sawdust for cellulose hydrolysis. Nig. J. Microbiol., 15: 97-102.
18. Hancock, D.D., T.E. Besser, D.H. Rice, E.D. Ebel, D.E. Herriott and L.V. Carpenter, 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA Preventive Vet. Med., 35: 11-19.
19. Baloch, A.B., X. Xia and S.A. Sheikh, 2015. Proximate and mineral compositions of dried cauliflower (*Brassica oleracea* L.) grown In Sindh, Pakistan. J. Food Nutr. Res., 3: 213-219.
20. Onyeze, R.C., G. T. Onah and O.C. Eluke, 2013. Bacterial contaminants associated with commercial poultry feeds in Enugu Nigeria. Int. J. Life Sci. Biotechnol. Pharm. Res., 2: 432-437.
21. Esimone, C., C. Nworu and G. Harrison, 2010. Antibiogram and plasmid profile of some multi-antibiotics resistant urinopathogens obtained from local communities of Southeastern Nigeria. Ibnosina J. Med. Biomed. Sci., 2: 152-159.