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Prevalence and antimicrobial resistance profiles of faecal *Escherichia coli* isolates from local chickens in Plateau State, Nigeria

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Abstract:

Background: Poultry is a profitable business in Nigeria, with economic benefits to families and communities involved in this type of agriculture. However, infection of poultry birds by *Escherichia coli* can, in addition to causing mortality, results in reduction of egg production, with depletion of protein (egg and meat) and subsequent reduction in market value, consumer supply, cost of veterinary care, and medicines. The objectives of this study are to determine the prevalence and antimicrobial resistance profiles of faecal *E. coli* isolates from local chickens (*Gallus domesticus*) in Plateau State, northcentral Nigeria.
Methodology: This was a descriptive cross-sectional study of 540 local chickens for faecal carriage of *E. coli*, randomly selected from 9 local government areas (LGAs) (60 per LGA) in the 3 senatorial districts (180 per senatorial district) of Plateau State, Nigeria. Faecal samples were collected from the chickens for culture isolation and identification of *E. coli* using conventional microbiological methods. The isolates were confirmed by Vitek® 2 compact machine and PCR amplification of 16S rRNA gene. Antibiotic susceptibility test of 37 distinct *E. coli* strains to selected antibiotics (ampicillin, ampicillin/sulbactam, piperacillin, cefazolin, cefepime, ceftriaxone, ceftazidime, cefoxitin, ertapenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin and trimethoprim/sulfamethoxazole) was performed by the Vitek® 2 and read using the web system application. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 and R Commander version 2.9-1.
Results: The overall prevalence of faecal *E. coli* carriage among the chickens was 65.0% (351/540), with highest prevalence in Central Plateau senatorial district (76.1%, 137/180), which was significantly higher than Northern Plateau (62.2%, 112/180) and Southern Plateau (56.7%, 102/180) (x^2=15.873, p=0.0004). The prevalence of *E. coli* was highest in Pankshin (86.7%, 52/60) and Mangu (85.0%, 51/60) LGAs and this was significantly higher than in Bokkos (56.7%, 34/60) (x^2=18.761, p<0.000) and other LGAs. The antibiotic susceptibility of 37 distinct strains of *E. coli* showed that 64.9% (n=24) were resistant to at least one antibiotic, with the highest resistance rate being to trimethoprim-sulfamethoxazole (51.4%, n=19), ampicillin (48.7%, n=18), and piperacillin (43.2%, n=15). Multi-drug resistance (resistance to three or more antibiotic classes) was observed in 35.1% (n=13) of the *E. coli* strains. The multiple antibiotic resistance (MAR) index ranged from 0.06 (resistance to one antibiotic) to 0.76 (resistance to 13 antibiotics tested).
Conclusion: The results of this study provide evidence that resistance to multiple antibiotics is widespread among faecal *E. coli* isolates from local chickens in Plateau State, Nigeria, and thus poses potential risks for human infections with MDR *E. coli*.

Keywords: local chicken; faecal carriage; *Escherichia coli,* multi-drug resistance; zoonosis

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Profils de prévalence et de résistance aux antimicrobiens des isolats fécaux d'Escherichia coli provenant de poulets locaux dans l'État du Plateau, Nigeria

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Contexte: La volaille est une activité rentable au Nigeria, avec des avantages économiques pour les familles et les communautés impliquées dans ce type d'agriculture. Cependant, l'infection des volailles par Escherichia coli peut, en plus de provoquer la mortalité, entraîner une réduction de la production d'œufs, avec un appauvrissement des protéines (œufs et viande) et une réduction ultérieure de la valeur marchande, de l'approvisionnement des consommateurs, du coût des soins vétérinaires et des médicaments. Les objectifs de cette étude sont de déterminer la prévalence et les profils de résistance aux antimicrobiens des isolats fécaux d'*E. coli* provenant de poulets locaux (*Gallus domesticus*) dans l'État du Plateau, au centre-nord du Nigéria.
Méthodologie: Il s'agissait d'une étude transversale descriptive portant sur 540 poulets locaux pour le transport fécal d'E. coli, sélectionnés au hasard dans 9 zones de gouvernement local (LGA) (60 par LGA) dans les 3 districts sénatoriaux (180 par district sénatorial) du Plateau État, Nigéria. Des échantillons de matières fécales ont été prélevés sur les poulets pour l'isolement des cultures et l'identification d'*E. coli* à l'aide de méthodes microbiologiques conventionnelles. Les isolats ont été confirmés par la machine compacte Vitek® 2 et par amplification PCR du gène de l'ARNr 16S. Test de sensibilité aux antibiotiques de 37 souches distinctes d'*E. coli* à des antibiotiques sélectionnés (ampicilline, ampicilline-sulbactam, pipéracilline, céfazoline, céfépime, ceftriaxone, ceftazidime, céfoxitine, ertapénème, méropénem, amikacine, gentamicine, tobramycine, ciprofloxacine, lévofloxacine, nitrofurantoïne et triméthoprime-sulfaméthoxazole) a été réalisée par le Vitek® 2 et lue à l'aide de l'application du système Web. L'analyse des données a été réalisée à l'aide du progiciel statistique pour les sciences sociales (SPSS) version 20.0 et de R Commander version 2.9-1.
Résultats: La prévalence globale du portage fécal d'*E. coli* parmi les poulets était de 65,0% (351/540), avec une prévalence plus élevée dans le district sénatorial du Plateau Central (76,1%, 137/180), qui était significativement plus élevée que dans le Plateau Nord (62,2%, 112/180) et Plateau Sud (56,7%, 102/180) (x^2=15,873, p=0,0004). La prévalence d'*E. coli* était la plus élevée dans les LGA de Pankshin (86,7%, 52/60) et de Mangu (85,0%, 51/60), et elle était significativement plus élevée qu'à Bokkos (56,7%, 34/60) (x^2=18,761, p<0,000) et autres LGA. La sensibilité aux antibiotiques de 37 souches distinctes d'*E. coli* a montré que 64,9% (n=24) étaient résistantes à au moins un antibiotique, le taux de résistance le plus élevé étant celui du triméthoprime-sulfaméthoxazole (51,4%, n=19), de l'ampicilline (48,7%, n=18) et pipéracilline (43,2%, n=15). Une multi-résistance aux médicaments (résistance à trois classes d'antibiotiques ou plus) a été observée chez 35,1% (n=13) des souches d'*E. coli*. L'indice de résistance multiple aux antibiotiques (MAR) variait de 0,06 (résistance à un antibiotique) à 0,76 (résistance à 13 antibiotiques testés).
Conclusion: Les résultats de cette étude prouvent que la résistance à plusieurs antibiotiques est répandue parmi les isolats fécaux d'*E. coli* provenant de poulets locaux dans l'État du Plateau, au Nigeria, et présente donc des risques potentiels d'infections humaines par *E. coli* MDR.

Mots-clés: poulet local; transport fécal; *Escherichia coli*; la multirésistance aux médicaments; zoonose

Introduction:

Escherichia coli belongs to the *Enterobacteriaceae* family, which cause infection that poses or constitutes great hazard to the poultry industry causing loss of weight, reduction of egg production and high mortality (1). All over the world, *E. coli* infection is one of the serious problems that cause great threat to the profitability of avian enterprises. *E. coli* in poultry intestine is a member of the normal microbiota but the colonization of the respiratory tract by pathogenic *E. coli* strains is associated with extraintestinal disease which results in morbidity and mortality of poultry by causing septicaemia (2). Although *E. coli* is a normal flora inhabiting the intestinal tract of birds, under the influence of predisposing factors such as inadequate and faulty ventilation, overcrowding, hunger, thirst, extremes of temperatures and low vitality, high mortality during rearing, reduced weight gain and condemnation of birds at the time of slaughter (1).

Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with air sacculitis and associated pericarditis, perihepatitis and peritonitis being most typical (1). Naturally, *E. coli* infected chicks present with clinical signs such as loss of appetite, cyanosis, tendency to huddle respiratory distress, depression, reduction of weight gain, dropped wings, closed eyes, and labored breathing (1). Certain strains of *E. coli* have virulence properties associated with host tissue colonization, production of toxins, iron uptake systems, defensins and serum resistance, though the pathogenesis of colibacillosis is not completely understood (2).

Local chickens are free range birds which may serve as a reservoir or carrier of pathogenic *E. coli* to the environments, poultry and households. Due to increasing demand for egg and meat products in the poultry sector which is among the fastest growing agro-based industries worldwide, disease burden has however, remained a great challenge in poultry production (3). The majority of these bacteria do not cause disease because they are environmental and normal flora. However, with the estimated bacteria species reaching about 10^{30} worldwide, it is important to identify and differentiate those species that are pathogenic, particularly from a medical and public health perspective (4). However, contamination of food with pathogenic *E. coli* species can cause serious foodborne illness in humans (4).

Antibiotic resistance is a growing global health concern with the huge societal risk

of reverting to pre-antibiotic era if not addressed. By 2050, it is estimated that death from from an antibiotic-resistant infection will occur in every three seconds (5). Between the rise in antibiotic-resistant infections and the 90 billion tons of chicken meats that are produced worldwide annually, there is a well-documented connection and due to this connection, the World Health Organization (WHO) adopted the 'One Health' approach in 2017, which states that the health of people, animals, and the environment are inextricably related to one another. According to WHO, it is relevant to refer to 'One Health' approach when discussing antibiotic resistance, food safety, and the control of zoonoses (5). Infections caused by antibiotic-resistant bacteria have resulted in increased hospitalization rate and longer hospital stay for infected individuals (5).

The use of antibiotic as growth promotion has increased and has been linked to the development of antibiotic resistance among bacterial strains (4). The practice of administering sub-therapeutic doses of antibiotics to livestock in preventing disease did not only increase resistance in bacteria found in animals, but also in humans. As a result of farming practices, workers and consumers may be exposed to antibiotic-resistant bacteria. Poultry farm workers and families living on farms using antibiotics in the feed including neighboring families, have an elevated risk of exposure to antibiotic-resistant *E. coli*. Consumers that purchase poultry products which utilize antibiotics in production may also be exposed through cross-contamination from raw meat on surfaces and consumption of undercooked meat. Antibiotic resistance genes are able to move horizontally, especially through conjugative gene transfer, to other bacteria (6). It has been suggested in several studies that *E. coli*, specifically, to human health has particular relevance because is able to transmit from retail meat to people and ultimately be source of urinary tract infections (7).

The emergence of resistance has the potential to impact on the treatment and management of infectious diseases in both animals and humans. This increasing resistance has received considerable national and international attention (1). This research aimed to determine the occurrence and antimicrobial resistance profiles of pathogenic *E. coli* in cloacal swabs of local chickens (*Gallus domesticus*) in Plateau State, Nigeria.

Materials and method:

Study area:

This study was carried out at the Antimicrobial Resistance Laboratory and Biotechnology Division, National Veterinary Research Institute, Vom, Plateau State. Plateau is a State in northcentral Nigeria with Jos as the administrative capital. It has an area of 26,899 km^2 is located between latitude 80°24'N and longitude 80°32' and 100°38' East. The State is bordered to the north by Kaduna and Bauchi States, with Benue State on the southern border and flanked on the West and East sides by Nasarawa and Taraba States respectively. Presently, the State has 17 Local Government Areas (LGAs) which include; Jos-North, Jos-East, Jos-South, Bassa, Riyom, Barkin Ladi, Bokkos, Mangu, Pankshin, Kanke, Kanam, Langtang-North, Langtang-South, Wase, Mikang, Shendam and Quanpan.

Plateau State derives its name from the geographical landscape that predominates this part of the country which is often referred to as the Jos Plateau. The economic potential of the State is mainly agrarian with over 70% of its population engaging in agriculture or agricultural related areas. The study was carried out in 3 Senatorial Zones of Plateau State, Nigeria (Fig 1).

Study design and sample size determination:

This was a descriptive cross-sectional study of poultry birds for faecal carriage of *E. coli*. The sample size for the study was determined using the formula, $N=Z^2pq/L^2$, where N is the sample size, Z is the level of confidence for two-tailed tests at 95% (=1.96), p is the reported prevalence of *E coli* which was 13.4% from Mude et al., (9), and L is the allowable error (precision) of 5% (=0.05). This gave the sample size of 178, which was adjusted to 180.

Source: (8)

Fig 1: Map of Plateau State showing the study sites in the three Senatorial Districts

Method of poultry sampling and cloacal sample collection:

Three local government areas (LGAs) in each of the 3 senatorial districts in Plateau State were selected by systematic random sampling technique. Sixty local chickens were randomly selected from each LGA, giving an overall total of 540 chickens, with 180 from each senatorial district. Cloacal swabs samples were collected from each local chicken using a sterile swab stick in buffered peptone water and transported in cold chain to the laboratory for analysis.

Culture of *Escherichia coli* from sample:

The procedure Feng et al., (10) was used for culture isolation of *E. coli*. The swabs were dipped into 10 ml buffered peptone water and incubated at 37°C for 24 hours after which 0.5 mL of the aliquots were dispensed into Ec broth and incubated at 37°C for 24 h, then a loop full of the aliquots were streaked on MacConkey agar plate (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. The growth was sub-cultured on Levine's Eosin-Methylene Blue Agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. Presumptive identification of *E. coli* was based on colonial morphology and cultural appearance (appeared as dark centered and flat, with or without the presence of green metallic sheen) and Gram-staining reaction.

Biochemical identification of *E. coli* isolates:

Suspected *E. coli* isolates were confirmed using conventional biochemical tests such as indole, methyl red, Voges-Proskauer (VP), citrate, and sugar fermentation reactions. Confirmed *E. coli* isolates were kept at 4°C in nutrient agar slants and skimmed milk for determination of antimicrobial susceptibility tests and further studies.

Molecular detection of *E. coli* by polymerase chain reaction (PCR) assay:

DNA extraction:

DNA extraction of phenotypically identified *E. coli* isolates was done using DNA extraction mini kit (Qiagen, USA). In brief, the pooled culture was placed into 1.5 Eppendorf tubes and centrifuged, the cell pellet was resuspended in phosphate buffer saline to a final volume of 200 µL. 20 µL of proteinase K and 200 µL buffer AL were added to the suspension and mixed by pulse-vortexing for 15 seconds. The tube containing the suspension was incubated at 56^0C for 10 mins and then centrifuged to remove drops from the inside of the lid. About 200 µL of ethanol (96-100%) was added to the suspension and mixed by pulse-vortexing, and then briefly centrifuged to remove drops from the inside of the lid.

The mixture was carefully applied to the QIAamp Mini spin column (in a 2mL collection tube) without wetting the rim. The cap

was closed and centrifuged at 6000 x g (8000 rpm) for 1min. The QIAamp Mini spin column was placed in a clean 2 mL collection tube and the tube containing the filtrate was discarded. The QIAamp Mini spin column was then carefully opened and 500 µL buffer AW1 was added without wetting the rim. The cap was closed and centrifuged at 600 x g (8000 rpm) for 1min. The QIAamp Mini column was placed in a clean 2 mL collection tube and the collection tube containing the filtrate was discarded. Again, the QIAamp Mini spin column was carefully opened and 500 µL buffer AW2 was added without wetting the rim. The cap was closed and centrifuge at full speed of 20,000 x g (14,000 rpm) for 3 mins. QIAamp Mini spin column was placed in a new 2ml collection tube and the old collection tube was discarded with the filtrate. It was then centrifuged at full speed for 1 min. The QIAamp Mini column was placed in another clean 1.5 mL microcentrifuge tube, the collection tube containing the filtrate was discarded. The QIAamp Mini column was carefully opened and 200 µL buffer AE was added and then incubated at room temperature (15-25^{0}C) for 1min and centrifuged at 6000 x g (8000 rpm) for 1 min, after which the QIAamp Mini column was discarded, and the filtrate is the DNA extract.

PCR amplification E. coli 16S rRNA gene:

About 5 µL of the DNA extract from each *E. coli* isolate were added to 20 µL of the master-mix which contains green Taq DNA polymerase, 2 x MM (12.5), nuclear free water (5.5), including specific target primers (forward primer ID *E. coli* 16S 11-F-5'-ATCAACC GAGATTCCCCCAGT-3'and reverse primer ID *E. coli* 16S 1338-R-5'-CACTATCGGTCAGTCAG GAG-3' with expected amplicon size of 401 bp. GenBank accession number NCTC-13846 was used as control.

The PCR reaction was caried in a thermal cycler with initial denaturation at 95^{o}C for 5 mins for one cycle and the second step involved denaturation for 35 cycles at 95^{o}C for 1 min, annealing at 50^{o}C for 50 seconds, and extension at 72^{o}C for 1 min, with a final extension at 72^{o}C for 10 minutes.

Agarose gel electrophoresis of PCR amplicons:

Approximately 15µL of the PCR amplicons of each isolate along with 5µL of the base pair DNA marker (New England Biolabs®) used as a molecular size marker, were loaded into different wells of prepared agarose gel into which 5µL ethidium bromide has been added. This was then carefully placed in electrophoresis tank and totally submerged in TBE buffer solution. The electrophoresis tank was closed, and the positive and negative terminals of the electrophoresis tank was connected to power source and run for 40 minutes at 80 volts. After the electrophoresis run, the amplicon bands were visualized and photographed using the Gel Documentation System (Bio Rad).

Antimicrobial susceptibility test of *E. coli* isolates by Vitek 2 system

Antibiotic susceptibility of confirmed *E. coli* isolates was performed with the VITEK® 2 compact machine (BioMerieux, USA). First, pure culture of each isolate was inoculated on Columbia sheep blood agar and incubated at 37°C for 24 hours. The Vitek® 2 compact cassette, Densicheck plus, saline dispenser, sterile swabs, polystyrene tubes on racks, lint-free wipes, pure culture plates of isolates were placed on a clean flat bench and all configuration options were set correctly. Inoculum suspension of the isolate was prepared and standardized to 0.5-0.63 McFarland standards using the Densicheck. About 145 µL of the suspension was transferred into 3 ml of saline, the test card (Gram-negative) was placed in the appropriate slots on the cassette and the cassette information was entered into FLEX prep view in the Vitek® 2 system web application. The cassette was then placed into the chamber and fill door closed to begin the filling process.

After completion of the fill cycle, the cassette was removed from the filler station and loaded into the load/unload station. Once loaded, the instrument bar code reader scans the test cards and cassette bar code. During the test card processing, the instrument unloads the test cards from the cassette and placed them into the carousel (in the incubator). After scanning, the cards are removed, and results read as susceptible (S), intermediate (I) or resistant (R) using the Vitek® 2 system web application.

Statistical analysis:

The prevalence data for *E. coli* were presented as percentage frequency and analysed using the Statistical Package for the Social Sciences (SPSS) Version 20.0 and R Commander version 2.9-1.

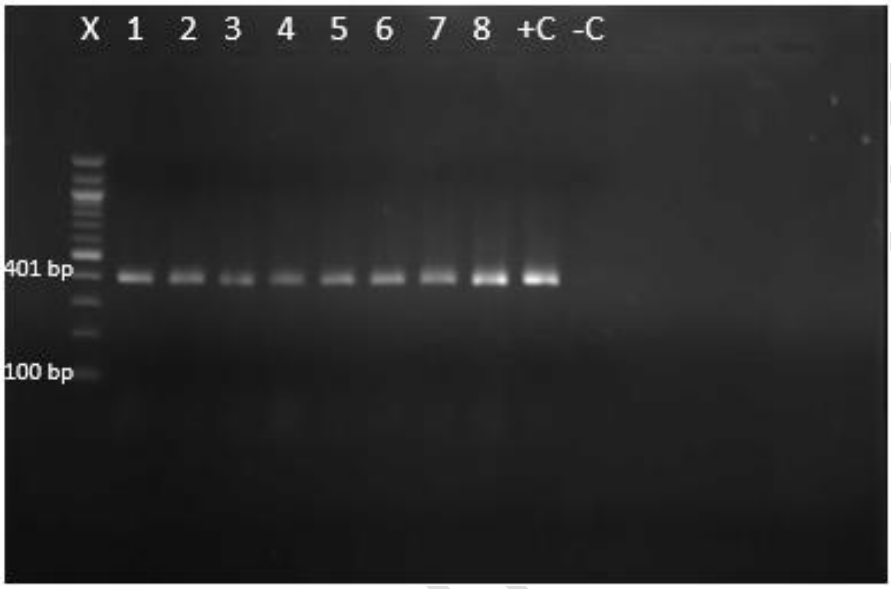
Results:

Prevalence of faecal carriage of *E. coli* in the chickens:

Of the 540 cloacal swab samples collected from 540 local chickens, *E. coli* was isolated and confirmed in 351 samples (Fig 1), giving an overall prevalence of *E. coli* carriage of 65.0% in the study. The prevalence of *E. coli* based on senatorial zones shows that Central Plateau has the highest prevalence rate of 76.1% (137/180), followed by Northern Plateau (62.2%, 112/180) and Southern Plateau, with the lowest prevalence of 56.7% (102/180). The statistics show the prevalence of faecal *E. coli* in the chickens to be significantly higher in Central Plateau than Southern

and Northern Plateau senatorial districts (x^2= 15.873, p=0.0004) (Table 1).

The prevalence of *E. coli* based on LGAs shows that Pankshin with 86.7% (52/60) had the highest prevalence, followed by Mangu with 85.0% (51/60), Riyom with 65.0% (39/ 60), Shandam and Barkin Ladi with 63.3% (38/60) each, Jos North with 58.3% (35/60), Mikang and Bokkos with 56.7% (34/60) each, and Qua'anpan with the least occurrence of 50.0% (30/60) (Table 1). In Central Plateau district, the prevalence of faecal *E. coli* was significantly higher in Pankshin and Mangu compared to Bokkos LGA (x^2=18.761, p<0.0 00) and to other LGAs in Northern and South-ern Plateau senatorial districts.



Lane X: Molecular size marker 100 bp DNA Ladder, Lane 1 - 8: Positive samples, Lane +C: Positive control (reference strains of NCTC-11954) and Lane -C: Negative control (Nuclease free water).
All the selected isolates showed molecular relatedness with the positive control as seen in the bands produced after electrophoresis. All the samples ran equal distances with the control showing that the lanes 1-8 contained similar DNA sequence as the positive control (Lane +C).

Fig 1: Agarose gel electrophoresis of 16S rDNA amplicon of representative *Escherichia coli* isolates

Table 1: Prevalence of *E. coli* isolates in chickens based on Senatorial Districts and LGA of Plateau State, Nigeria

Senatorial districts	**⁺Number positive by Senatorial district (%)**	**LGAs**	**Number positive By LGA (%)**	**X^2**	**p-value**
Central Plateau (n=180)	137 (76.1)	Bokkos (n-60)	34 (56.7)	18.761	<0.000***
		Mangu (n=60)	51 (85.0)		
		Pankshin (n=60)	52 (86.7)		
Southern Plateau (n=180)	102 (56.7)	Qua'anpan (n=60)	30 (50.0)	2.172	0.338
		Mikang (n=60)	34 (56.7)		
		Shandam (n=60)	38 (63.31)		
Northern Plateau (n=180)	112 (62.2)	Jos North (n=60)	35 (58.3)	0.615	0.735
		Barkin Ladi (n=60)	38 (63.31)		
		Riyom (n=60)	39 (65.0)		
Total (n=540)	**351 (65.0)**	**Total (n=540)**	**351 (65.0)**		

⁺ = statistically significant difference in prevalence with respect to senatorial district (x^2=15.873, p=0.0004); *** = statistically significant difference in prevalence with respect to LGA in Central Plateau

Antimicrobial resistance profiles of *E. coli* isolates:

The phenotypic antimicrobial resistance of 37 distinct *E. coli* strains is as shown in Table 2 and Fig 1. The *E. coli* strains were resistant to trimethoprim-sulfamethoxazole (51.4%, n=19), ampicillin (48.7%, n=18), and and piperacillin (43.2%, n=15) but highly sus-

ceptible to cefoxitin, ertapenem, meropenem and amikacin (100.0%, n=37); ciprofloxacin, levofloxacin and nitrofurantoin (91.9%, n=34); cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin, and tobramycin (89.2%, n=33); and ampicillin-sulbactam (75.7%, n=28). Intermediate resistance was observed for few of the antibiotics tested, ranging from 2.7% (n=1) to 5.4% (n=2), except for ampicillin-sulbactam with 43.2% (n=6).

Table 2: Antibiotic susceptibility of 37 *Escherichia coli* strains isolated from cloacal of local chickens in Plateau State, Nigeria

Antimicrobial	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampicillin	18 (48.7)	1 (2.7)	18 (48.7)
Ampicillin-sulbactam	3 (8.1)	6 (43.2)	28 (75.7)
Piperacillin	16 (43.2)	2 (5.4)	19 (51.4)
Cefazolin	4 (10.8)	0	33 (89.2)
Cefoxitin	0	0	37 (100.0)
Ceftazidime	4 (10.8)	0	33 (89.2)
Ceftriaxone	4 (10.8)	0	33 (89.2)
Cefepime	4 (10.8)	0	33 (89.2)
Ertapenem	0	0	37 (100.0)
Meropenem	0	0	37 (100.0)
Amikacin	0	0	37 (100.0)
Gentamicin	4 (10.8)	0	33 (89.2)
Tobramycin	2 (5.4)	2 (5.4)	33 (89.2)
Ciprofloxacin	3 (8.1)	0	34 (91.9)
Levofloxacin	2 (5.4)	1 (2.7)	34 (91.9)
Nitrofurantoin	1 (2.7)	2 (5.4)	34 (91.9)
Trimethoprim-sulfamethoxazole	19 (51.4)	0	18 (48.7)

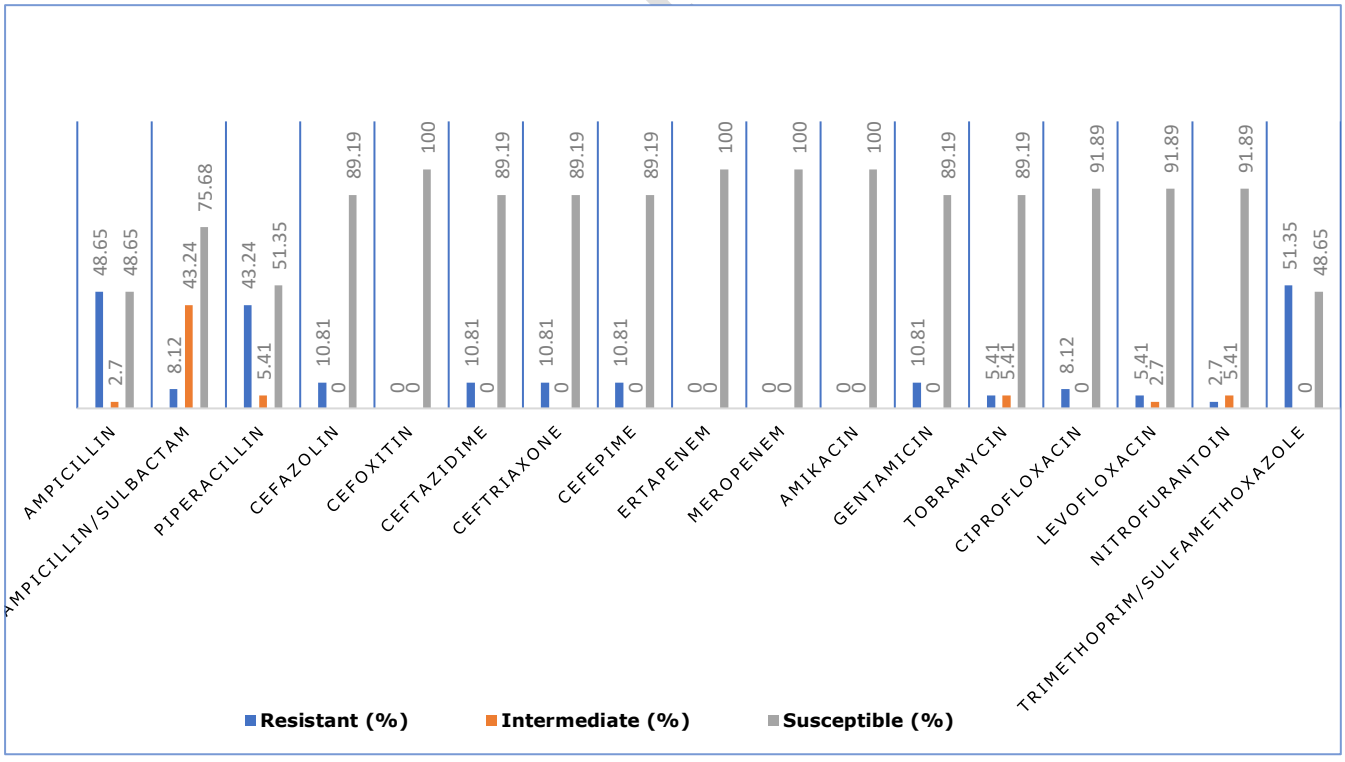


Fig 1: Antibiotics susceptibility of 37 *Escherichia coli* strains from local chickens in Plateau State, Nigeria

Multi-drug resistance and multiple antibiotic resistance index of *Escherichia coli* strains: The multiple antibiotics resistance (MAR) index, as shown in Table 3 and Fig 2, ranged from 0.06 (resistant to one antibiotic) to 0.76 (resistant to thirteen antibiotics) and the overall prevalence of multi-drug resistant (MDR) (resistance to two or more different classes of antimicrobials) with 35.1% (13/37).

Table 3: Antibiotic resistance profile and multiple antibiotic resistance index of 37 *Escherichia coli* strains isolates from cloacal of local chickens in Plateau State, Nigeria

S/NO	*Escherichia coli* code	Antibiotics resistant profile	Number of antibiotics	MARI
1	ASTGN9-1		0	0
2	ASTGN130-1		0	0
3	ASTGN156-1		0	0
4	ASTGN148-1		0	0
5	ASTGN292-1		0	0
6	ASTGN253-1		0	0
7	ASTGN379-1		0	0
8	ASTGN41-1		0	0
9	ASTGN113-1		0	0
10	ASTGN215-1		0	0
11	ASTGN211-1		0	0
12	ASTGN128-1		0	0
13	ASTGN249-1		0	0
14	ASTNG181-1	TrS	1	0.06
15	ASTGN90-1	TrS	1	0.06
16	ASTGN78-1	TrS	1	0.06
17	ASTGN2-1	TrS	1	0.06
18	ASTGN76-1	TrS	1	0.06
19	ASTGN395-1	Amp, Pip	2	0.12
20	ASRGN334-1	Amp, Pip	2	0.12
21	ASTGN122-1	Amp, Pip	2	0.12
22	ASTGN391-1	Amp, Cip	2	0.12
23	ASTGN31-1	Ntf, TrS	2	0.12
24	ASTGN387-1	Amp, TrS	2	0.12
25	ASTGN97-1	Amp, Pip, TrS	3	0.18
26	ASTNG136-1	Amp, Pip, TrS	3	0.18
27	ASTNG275-1	Amp, Pip, TrS	3	0.18
28	ASTGN30-1	Amp, Pip, TrS	3	0.18
29	ASTGN46-1	Amp, Pip, TrS	3	0.18
30	ASTGN58-1	Amp, Pip, TrS	3	0.18
31	ASTNG316-1	Amp, AmpS, Pip, TrS	4	0.24
32	ASTGN359-1	Amp, Pip, Gn, TrS	4	0.24
33	ASTGN384-1	AmpS, Pip, Gn, TrS	4	0.24
34	ASTGN195-1	Amp, Pip, Cef, Cefz, Cefx, Cep	6	0.35
35	ASTGN236-1	Amp, Pip, Cef, Cefz, Cefx, Cep,	6	0.35
36	ASTGN149-1	Amp, AmpS, Pip, Cf, Cefz, Cefx, Cep, Gn, Tbr, Cip, Levf, TrS	12	0.71
37	ASTGN154-1	Amp, AmpS, Pip, Cf, Cefz, Cefx, Cep, Gn, Tbr, Cip, Levf, Ntf, TrS	13	0.76

Amp: ampicillin; AmpS: Ampicillin/Sulbactam; Pip: Piperacillin; Cip: ciprofloxacin; Cefx: ceftriaxone; Cef: Cefazolin; Cefz: Ceftazidime; Cep: Cefepime; Gn: Gentamicin; TrS: Trimethoprim/ sulfamethoxazole; Tbr: Tobramycin; Levf: Levofloxacin; Ntf: Nitrofurantoin

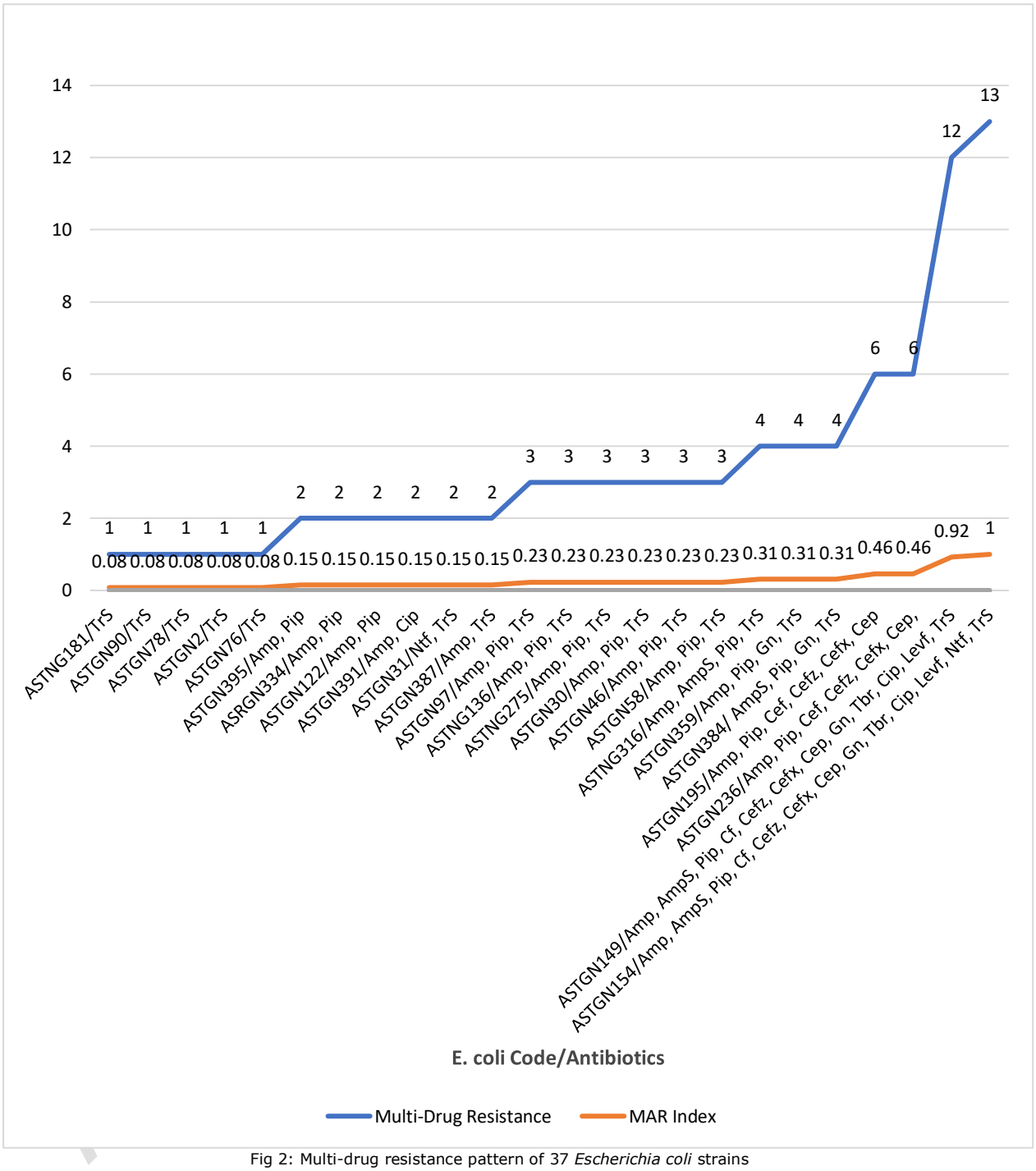


Fig 2: Multi-drug resistance pattern of 37 *Escherichia coli* strains

Discussion:

The early identification of carrier animals and information on the shedding state are crucial to prevent the spread of infection to animals and humans. It is a generally accepted opinion that the food chain has been recognized as one of the main passages for the transfer of antibiotic resistant bacteria between human and animal populations (11). The prevalence of 65.0% faecal carriage of *E. coli* in local chickens in our study is higher than the 50.0% reported in Zaria, Nigeria (12), but lower than the prevalence of 80.0% reported in a study conducted in Ghana by Adzitey et al., (13). Local chickens in Nigeria are frequently raised under free-range conditions with little care, exposing them to environmental contaminants and increasing the risk of bacterial infections (14). This may explain why local chickens in this study had a high prevalence of

faecal *E. coli* carriage. Local chickens in Nigeria are fed on food scraps, grass, maggots from cow dung, and other environmental waste, which could expose them to pathogenic bacteria like *E. coli* (15). They are also not routinely vaccinated or put on antibiotic medications.

This high prevalence (65.0%) of *E. coli* in this study is significant because of its pervasiveness and highly promiscuous nature for antimicrobial resistomes (16), which makes it a tool for the spread of antimicrobial resistant genes in the food chain and the environment. Due to the prevalence of resistant strains or residues in humans, the overall effect is that humans do not respond to antimicrobial treatments. Given that the local chickens are free range and in close proximity to other animals and the environment, the high rate of antibiotic resistance seen in this study may be the result of the indiscriminate use of antibiotics. According to reports, rather than being utilized to encourage growth in animals, antibiotics are primarily employed as preventative medicine and for the treatment of sick animals. Most farmers often fail to observe periods of withdrawal when giving antibiotics or other medications to their livestock and they are more likely to become resistant to antibiotics and to accumulate antimicrobials in their muscle tissues as an outcome.

The *E. coli* isolates were highly resistant to trimethoprim-sulfamethoxazole (51.4%) followed by ampicillin (48.7%), and piperacillin (43.2%) followed by low resistance to cefazolin, ceftazidime, ceftriaxone, cefepime and gentamicin (10.1%); ampicillin-sulbactam and ciprofloxacin (8.1%); tobramycin and levofloxacin (5.4%), with the least resistance to nitrofurantoin (2.7%), but susceptible to cefoxitin, ertapenem, meropenem and amikacin (100%); ciprofloxacin, levofloxacin and nitrofurantoin (91.9%); cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin and tobramycin (89.2%); and ampicillin-sulbactam (75.7%). Intermediate resistance was observed for few of the antibiotics examined, ranging from 2.7% to 5.4%, except for ampicillin-sulbactam with 43.2%. These findings are similar to those of the study by Vranic et al., (17) which showed highest resistance to trimethoprim-sulfamethoxazole (40.9%) and ampicillin (82.8%).

In this study, the multiple antibiotics resistance (MAR) index ranged from 0.06 (resistant to one antibiotic) to 0.76 (resistant to 13 antibiotics) and the study recorded multidrug resistance (MDR), that is resistance to 2 or more different classes of antimicrobials (18) among the isolates. Ampicillin, ampicillin-sulbactam, piperacillin, ciprofloxacin, ceftriaxone, cefazolin, ceftazidime, cefepime, gentamicin, trimethoprim-sulfamethoxazole, tobramycin, levofloxacin, and nitrofurantoin were all completely ineffective invitro against *E. coli* isolates in the study. This level of antibiotic resistance may have been brought on by unchecked and excessive use of antibiotics in the poultry (18-20). Availability and accessibility of antibiotics in poultry contribute to their overuse, and according to the research finding by Salihu (21), the resistance seen in *E. coli* isolated from local chickens was caused by the transfer of resistance gene(s) from another host in the same production setting. Due to instances of comparable antibiotic resistance genes being simultaneously recovered from human and poultry samples, the proximity of these poultry to home could constitute a serious threat to biosecurity (7). The absorption of resistant genes discovered in single and multiple size plasmids in *E. coli* isolates was the likely mechanism by which these resistances were acquired.

The World Health Organization (WHO) recognized antibiotic resistance as one of the greatest threats to public health because of its potential impact on health outcomes globally as more bacteria develop antibiotic resistance (22). According to a United Kingdom (UK) assessment, bacterial infections that are resistant to antibiotics could kill over 10 million people by the year 2050 (23). It is worrisome that most of these resistant bacteria are zoonotic, and infections triggered by these pathogens can be difficult to treat because previously potent drugs become less efficacious against the same pathogen. Environmental contamination by antibiotic residue and AMR genes has been shown to be a major driver of AMR in livestock because up to 70% of antimicrobials administered to livestock has been shown to be released as unmetabolized agents (24).

Conclusion:

Since many isolates in this study were resistant to two or more drugs, it is likely that the local chicken population is home to *E. coli* strains that are multidrug resistant. Therefore, local poultry might act as a conduit for MDR transmission to humans. Furthermore, the environment, farms, and live bird markets in areas with low levels of biosecurity may have a significant impact on the spread of MDR *E. coli* among animals and humans through their network.

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Contributions of authors:

CGA was the principal investigator who conceived the idea of the study; CGA, AC

and JFN designed the study; CGA and ECSO carried out sampling and bacteriological assays; CGA, KNA, BJA and DC carried out the microbiological analysis on PCR and Vitek-2; CGA, AC, JFN, MM and KNA wrote the manuscript and were responsible for the final editing of the manuscript. All authors approved the final manuscript submitted for publication.

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References:

1. Abd El Tawab, A., Ammar, A., Nasef, S., and Reda, R. Prevalence of *E. coli* in diseased chickens with its antibiogram pattern. Benha Vet Med J. 2015; 28 (2): 224–230. https://doi.org/10.21608/bvmj.2015.32507
2. Coura, F. M., Diniz, S. A., Silva, M. X., et al. Phylogenetic Group of *Escherichia coli* Isolates from Broilers in Brazilian Poultry Slaughterhouse. Sci World J. 2017; https://doi.org/10.1155/2017/5898701
3. Abdu, H. U., and Ibrahim, A. Isolation and Characterization of Multidrug-resistant *Escherichia coli* from Poultry Litter samples from Selected Farms in Kano metropolis, Nigeria. Nig J Microbiol. 2021 35 (1): 5623- 5629.
4. Spitzer, H. An Analysis of Bacterial Contamination of Chicken Eggs and Antimicrobial Resistance. College of Saint Benedict and Saint John's University Digital Com. 2015
5. Sanchez, H. M., Whitener, V. A., Thulsiraj, V., et al. Antibiotic resistance of *Escherichia coli* isolated from conventional, no antibiotics, and humane family owned retail broiler chicken meat. Animals. 2020; 10 (12): 1–17. https://doi.org/10.3390/ani10122217
6. McInnes, R. S., McCallum, G. E., Lamberte, L. E., and van Schaik, W. Horizontal transfer of antibiotic resistance genes in the human gut microbiome. Curr Opin Microbiol. 2020; 53: 35–43. doi:10.1016/j.mib.2020.02.002.
7. Johnson, J. R., Porter, S. B., Johnston, B., et al. Extraintestinal pathogenic and antimicrobial resistant *Escherichia coli*, including sequence type 131 (ST131), from retail chicken breasts in the United States in 2013. J Appl Environ Microbiol. 2017; 83 (6): e02956-16 doi:10.1128/AEM.02956-16
8. Chollom, C. S., Osawe, S., Lar, P., Egah, D. Z., Mamman, I., and Abimiku, A. Analysis of Reported Cases of Lassa Fever in Plateau State and the Need for Strategic Action Plan. Int J Trop Dis Health. 2016; 13 (2): 1-7. doi:10.9734/IJTDH/2016/20568
9. Mude, S., Thomas, N., Kemal, J., and Muktar, Y. Cloacael Carriage and Multidrug Resistance *Escherichia coli* O157:H7 from Poultry Farms, Eastern Ethiopia. J Vet Med. 2017; 2: 1-9. https://doi.org/10.1155/2017/8264583
10. Feng, P., Weagant, S. D., Grant, M. A., and Burkhardt, W. Enumeration of *Escherichia coli* and the coliform bacteria. Bacteriological Analytical Manual (BAM), Chapter 4: 2017. https://www.fda.gov/food/laboratory-methods-food/bam-4-enumeration-escherichia-coli-and-coliform-bacteria.
11. Rega, M., Andriani, L., Poeta, A., Bonardi, S., Conter, M., and Bacci, C. The Pork Food Chain as a Route of Transmission of Antimicrobial Resistant *Escherichia coli*: A Farm-to-Fork Perspective. Antibiotics. 2023. doi: 10.3390/antibiotics12020376
12. Ejeh, F. E., Lawan, F. A., Abdulsalam, H., Mamman, P. H., and Kwanashie, C. N. Multiple antimicrobial resistance of *Escherichia coli* and Salmonella species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria. Sokoto J Vet Sci. 2017; 15 (3): 45 – 53 doi:10.4314/sokjvs.v15i3.7
13. Adzitey, F., Assoah-Peprah, P., Teye, G. A., Somboro, A. M., Kumalo, H. M., and Amoako, D. G. Prevalence and Antimicrobial Resistance of *Escherichia coli* Isolated from Various Meat Types in the Tamale Metropolis of Ghana. Int J Food Sci. 2020; 12: 8877196 doi:10.1155/2020/8877196.
14. Geidam, Y. A., Ambali, A. G., and Onyeyili, P. A. Detection and antibiotic sensitivity pattern of avian pathogenic *Escherichia coli* strains among rural chickens in the arid region of north-eastern Nigeria. Vet World, 2012; 5 (6): 325–329.
15. Amadi, V. A., Watson, N., Onyegbule, O. A., et al. Antimicrobial resistance profiles of *Escherichia coli* recovered from feces of healthy free-range chickens in Grenada, West Indies. Int J Curr Microbiol Appl Sci. 2015; 4 (6): 168175
16. Nhung, N. T., Cuong, N. V., Thwaites, G., and Carrique-Mas, J. Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia: A Review. Antibiotics (Basel). 2016; 5 (4): 37 doi:10.3390/antibiotics5040037
17. Vranic, S. M., and Uzunovic, A. Antimicrobial resistance of *Escherichia coli* strains isolated from urine at outpatient population: a single laboratory experience. Mater Sociomed. 2006; 28 (2): 121-124. doi:10.5455/msm.2016.28.121-124.
18. Velge, P., Cloeckeart, A., and Barrow, P. Emergence of *Salmonella* epidemics: The problem related to *Salmonella enterica* serotype *enteritidis* and multiple antibiotic resistance in other major serotypes. J Vet Res. 2005; 36 (3): 267–288. doi:10.1051/vetres:2005005.
19. Nsofor, C. A., Iroegbu, C. U., Call, D. R., and Davies, M. A. The genetic relatedness of drug resistant *E. coli* isolates of human and animal origin in Nigeria. Int J Genet Mol Biol. 2013; 5 (3): 37–41. doi:10.5897/IJGMB2013.0077
20. Adeyanju, G. T., and Ishola, O. Salmonella and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo state, Nigeria. Springer plus. 2014. doi: 10.1186/2193-1801-3- 139.
21. Salihu, A. E., Onwuliri, F. C., and Mawak, J. O. Antimicrobial resistance profiles of *Salmonella gallinarum* isolates from free range chickens in Nasarawa state, Nigeria. Int J Bacteriol Res. 2014 2 (1): 19 - 27.
22. World Health Organization. Global Action Plan on Antimicrobial Resistance. Geneva: WHO. 2017.
23. O'Neill, J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. 2014; 1–16.
24. Anand Kumar, P. Antimicrobial Resistance (AMR) Surveillance Under One Health. In: Mothadaka, M. P., Vaiyapuri, M., Rao Badireddy, M., Nagarajrao Ravishankar, C., Bhatia, R., Jena, J. (eds). Handbook on Antimicrobial Resistance. Springer, Singapore. 2023: 1-15 doi:10.1007/978-981-16-9723-4_33-1