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Antimicrobial resistance profile of *Escherichia coli* species isolated from broiler chickens in the Southeastern Region of Nigeria

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Abstract

Poultry production plays an important role in meeting the national demand for animal protein. However, intensive poultry farming can facilitate the transmission and spread of pathogenic microorganisms. *Escherichia coli* is one of the bacteria species that is frequently associated with various avian diseases that result in considerable economic losses to the poultry industry in Nigeria. The extensive use of antimicrobial agents in poultry production has reportedly contributed to the emergence of multidrug-resistant *E. coli*, making infections increasingly difficult to treat, and raising concerns about the zoonotic transmission of antimicrobial resistance (AMR). Consequently, continuous surveillance of AMR in poultry populations is essential. This study determined the AMR profile of *E. coli* isolated from broiler chickens in Southeastern region of Nigeria. One hundred and twenty (120) cloacal swabs were collected from broilers in Anambra, Imo, Akwa Ibom and Rivers States of Nigeria. The *E. coli* was isolated by culture and confirmed biochemically. Antimicrobial susceptibility to ten antibiotics was determined by disc diffusion method. Results showed that *E. coli* was isolated from 56 (46.7%) of the cloacal swabs, composed of 10 (33.3%), 18 (60%), 12 (40.0%), and 16 (53.3%) from Anambra, Akwa Ibom, Imo and Rivers States, respectively. All the isolates were multidrug-resistant. The percentage of the isolates that were resistant to the following antibiotics was very high: Tetracycline – 100%, Streptomycin – 98.2%, Ceftriaxone – 92.9% and Sulphamethoxazole – 85.7%; and the lowest occurrence of resistance was recorded for Clindamycin – 1.8%. It was concluded that broiler chickens in the study area harboured multidrug-resistant *E. coli*, confirming them as reservoirs of antimicrobial resistance traits, which poses a public health risk through potential transmission to humans and other bacteria, and underscoring the need for continued AMR surveillance in poultry production.

Keywords: *Escherichia coli*; Occurrence; Poultry; Broiler chickens; Antimicrobial resistance; Southeastern region of Nigeria.

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Introduction

The increasing need for animal protein has led to intensive poultry production in most countries. Intensive poultry production, however, provides the optimum condition for the concentration and transmission of pathogens (Collins, 2008). This is because the crowding of birds in an enclosed environment is highly conducive for transmission of pathogenic organisms. Furthermore, genetic selection of birds for higher performance has left the birds' immune system less able to cope with infections. There is also higher degree of genetic uniformity in modern intensively farmed poultry, thus, making the spread of diseases easier (Delany, 2003). The economic and public health importance of these diseases in intensive poultry production system has been emphasized by several reports indicating the need for their prevention and control (Okoli, 2004; Okoli *et al.*, 2006; Chima *et al.*, 2011).

Among these diseases and disease causing agents of major economic and public health importance, *Escherichia coli* infection is about the earliest that birds are exposed to right from the hatchery (Gengerich, 2011). *E. coli* is a gram-negative bacterium that can cause various diseases in poultry. The organism is ubiquitous and could be isolated wherever faecal material from animals is found. For example, *E. coli* 0157 can normally be found in the gastro-intestinal system of a range of domestic animals including poultry (Ugwu *et al*., 2017). Thus, *E. coli* related diseases continue to be at or near the top of the list of diseases of broilers under intensive production systems (Gengerich, 2011).

The adverse economic effects of *E. coli* infections in poultry are specifically seen as poor laying performance, very high early mortalities, poor growth and viability in laying flocks, broiler units and starter chicks (Mmeremikwu, 2001; Okoli, 2004). *E. coli* infections are more prevalent in poor production environments, which are common with resource poor producers that constitute the bulk of poultry farmers in Nigeria (Okeudo, 2004).

Antimicrobial agents are used widely for maintenance of health and welfare of livestock as well as humans (Tamura, 2003). A major part of routine antimicrobial usage is for prevention of diseases, and this practice is currently an integral part of modern industrialized poultry production, to the extent that nearly all feeds for growing birds are supplemented with antimicrobials at various doses ranging from sub-therapeutic concentrations to full therapeutic doses (Wegener, 2012). The World Health Organization (WHO, 1997) and the Food and Agriculture Organization (FAO *et al.,* 2003a & b) estimated that antimicrobial use in food animals exceeds the use in humans worldwide. Again, most classes of antimicrobials used for humans are also being used in food animals, including the newest classes of drugs such as third- and fourth-generation cephalosporins, fluoroquinolones, glycopeptides and streptogramins (Aarestrup *et al.,* 2008).

The use and misuse of antimicrobials has been linked to the emergence and increasing prevalence of antimicrobial resistant bacteria (Tamura, 2003). The impact on human health has been a concern in recent decades. For example, some antimicrobial resistant pathogens such as the extended beta-lactamase producing *Salmonella* and *E. coli*, transmissible quinolone resistant *Salmonella* and *E. coli,* and animal-associated methicillin-resistant S*taphylococcus aureus*, which can cause infection in humans, have reportedly emerged in the food production chain (Aarestrup *et al.,* 2008). Their emergence has been associated with the use of antimicrobial agents in food animals, and this has led to the campaign for banning of the use of certain types of antimicrobials in food animals;

those that are considered critically important to human health (Xia *et al.,* 2010).

Understanding the zoonotic antimicrobial resistance (AMR) problem in its full complexity requires monitoring of antimicrobial usage and resistance in all relevant bacterial reservoirs and stages in the transmission route, and coherent analysis of the data i.e. "integrated monitoring" (Bager *et al.,* 1999; Okoli *et al.,* 2002; Okoli, 2006). For the purpose of intervention, there are usually multiple potential points of control that may be used, depending on the specific nature of the problem. Identifying and intervening at the most critical points of control requires a comprehensive assessment of the risks based on integrated monitoring, as well as good collaboration between all the stakeholders involved (Okoli *et al.,* 2005; WHO, 2005). Furthermore, since any antimicrobial use will exert selection pressure on exposed bacteria and may result in the development of resistance (Levy, 1997), it means that information needed for effective intervention will be required on the antimicrobial substance used, the mode of usage and the quantities used (Franklin *et al.,* 2001; Okoli *et al.*, 2002). Since these vary with different farms and farming systems, monitoring of individual farms could give more detailed insight into the exposure of the birds to antibiotic usage and the resistance level of the bacteria (Okoli *et al.,* 2002; Okoli, 2004).

Reports have shown that antimicrobial resistance in *E. coli* and other enteric bacteria like *E. faecalis* is usually mediated by the acquisition of one or several new genes, rather than by point mutation in existing genes (Lipsitch and Samore, 2002). This means that organisms or plasmid bearing new types of resistance must be acquired, generally as a consequence of cross transformation. Again, such organisms are not obligate organisms; therefore, much of their exposure to antimicrobials is during treatment directed at infections caused by unreported organisms (Halloran and Struchiner, 1991; Okoli, 2004). In these bacterial organisms, the increase in transmission of resistant strains is a consequence of successful treatment of infected host, which resulted in the eradication of susceptible pathogens that colonized and infected the host (Lipsitch and Samore, 2002).

In poultry production, monitoring of *E. coli* AMR is important since the organism (*E. coli*) is among the earliest organisms the birds are exposed to right from the hatchery and brooding period and are non-target organism in most enteric therapeutic interventions. *E. coli-based* AMR surveillance programmes may serve as an early warning system in the sense that even minor shifts in their antibacterial susceptibilities may be identified at the earliest stage. Interventions may thus be undertaken to limit further shifts in susceptibility or spread of resistance (Leegaard *et al.,* 2000). The present study determined the occurrence and antimicrobial resistance attributes of *E. coli* isolated from broilers in Southeastern region of Nigeria.

Materials and Methods

Study Areas: The study was conducted in four states (Anambra, Imo, Rivers and Akwa Ibom States) in the Southeastern Region of Nigeria (Figure 1). The area is located between latitude 5°00'N to 6°00'N of the equator and longitude 7°30'E to 8°30'E east of the Greenwich meridian (Figure I). For administrative purposes, Imo State is divided into 27 Local Government Areas (LGAs), which are further grouped into three senatorial or agricultural zones namely, Owerri, Orlu and Okigwe. Anambra State is divided into 21 LGAs further grouped into Anambra South, Central and North senatorial or agricultural zones. Rivers and Akwa Ibom States presently belong to the South-South geo-political zone of Nigeria. Rivers State is divided into 23 LGAs,

which are further grouped into three zones namely, Rivers southeast, west and east. Akwa Ibom State, on the other hand, is divided into 31 LGAs further grouped into Eket, Uyo and Ikot Ekpene senatorial zones.

Selection of Study Sites and Sample Collection: A two week preliminary survey of the study area was carried out to identify the study sites in each of the States. Three study sites were selected from each state to represent the three agricultural/senatorial zones, using urban centres with aggregates of animal healthcare practices and poultry farming activities (Okoli *et al.,* 2002 and 2005). One broiler farm was selected purposively from each of the three agricultural zones of each state, making a total of 3 farms per state and a total of 12 farms in the study area. From each of the 12 farms, 10 cloacal swabs from randomly selected chickens were collected, making a total of 120 samples.

Sampling: In each farm selected for the study, cloacal swab samples were collected with sterile cotton-tipped swab sticks from 10 adult chickens. The chickens were selected using a simple random sampling technique by catching two each at the four corners and the centre of the pen. The swab sticks were properly labeled and transported to the Imo State Environmental Protection Agency (ISEPA) Microbiology Laboratory in cold boxes within four hours of their collection. Samples from Rivers and Akwa Ibom States, which could not be transported to the laboratory within four hours, were stored in a refrigerator at 4^{o}C and transported to the ISEPA laboratory the following day in cold boxes. The owners of each farm sampled granted permission and consented to the sampling for research purposes, and the animals were handled humanely according to the procedures approved the Institutional Animal Ethics Committee of the Federal University of Technology, Owerri, Nigeria.

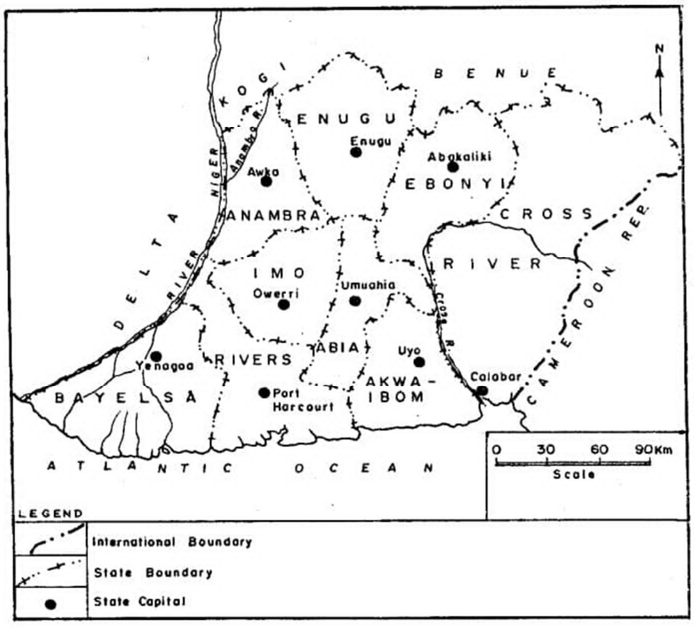


Figure 1. Part of the Nigerian map showing the study areas of Anambra, Imo, Rivers and Akwa Ibom States of Nigeria (Onweluzo and Eillita, 2003).

Culture and Isolation of *Escherichia coli:* The swab samples were each cultured on MacConkey Agar plates by touching the cotton-tipped swab stick on a corner of the agar surface. Each inoculum was spread using a sterile wire loop. The inoculated plates were incubated at 37°C for 24 hours. Colony characteristics of the organism (2- 4 mm diameters, opaque and convex with entire edge and pink on account of lactose fermentation) on the media were identified as presumptive *E. coli* (Ugwu *et al*., 2017). These pink colonies were sub-cultured onto Eosin Methylene Blue agar. The colonies with a metallic green sheen appearance with a flat surface were suspected to be *E. coli* and were stored in Nutrient Agar slants in Bijou bottles in the refrigerator for future use.

Antimicrobial Sensitivity/Susceptibility Test: Antimicrobial susceptibility testing was carried out with the *E. coli* isolates obtained, according to the procedure recommended by CLSI (2024). Antimicrobial susceptibility tests of the isolates were determined by the disc diffusion technique on Mueller-Hinton agar (CLSI, 2024). This was done against a panel of ten antibiotics which included Chloramphenicol (10 ug), Gentamycin (10 ug), Tetracycline (30 ug), Ciprofloxacin (5 ug), Cefotaxime (30 ug), Erythromycin (25 ug), Sulphamethoxazole (10 ug), Clindamycin (5 ug), Cefraxone (30 ug) and Streptomycin (30 ug) (Oxoid, England). The susceptibility of *E. coli* to the different antibiotics was determined by the zone of inhibition. Zone of inhibition or clearance was obtained by measuring the diameter of inhibition across each disc with a meter rule to the last whole millimetre. The isolates were classified as susceptible or resistant according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2024).

Data Analysis and Presentation

Data generated from the study were subjected to descriptive statistics using the SPSS software, and presented as frequencies or percentages of occurrence.

Results

Isolation rate of *E. coli* from Chicken in the Study Area: Out of the 120 samples collected, 56 samples were positive for *E. coli,* representing 46.7% of the samples tested (Table 1). Out of the 30 samples collected from each State, 10 (33.3%), 18 (60%), 12 (40.0%), and 16 (53.3%) were isolated from Anambra, Akwa Ibom, Imo and Rivers States, respectively. Akwa Ibom State had the highest occurrence (18 isolates), while Anambra State had the lowest occurrence (10 isolates) among the States studied.

Table 1. Occurrence of *Escherichia coli* in cloacal swabs of broiler chickens sampled in selected States in Southeastern Region of Nigeria.

States sampled	Number of broiler chicken sampled	Number positive for *E. coli*	Percentage positive for *E. coli*
Anambra	30	10	33.3%
Akwa Ibom	30	18	60.0%
Imo	30	12	40.0%
Rivers	30	16	53.3%
Total	**120**	**56**	**46.7%**

Table 2. Antibacterial resistance profile of the 56 *Escherichia coli* isolates obtained from cloacal swabs of broiler chickens sampled in selected states in Southeastern Region of Nigeria.

Antimicrobial agent	Number of resistant isolates out of the 56	Percentage of the isolates that were resistant
Chloramphenicol (10 µg)	36	64.3%
Ciprofloxacin (5 µg)	38	67.9%
Tetracycline (5 µg)	56	100%
Gentamicin (10 µg)	23	41.1%
Streptomycin (30 µg)	55	98.2%
Cefotaxime (30 µg)	45	80.4%
Erythromycin (25 µg)	44	78.6%
Sulphamethoxazole (10 µg)	48	85.7%
Clindamycin (5 µg)	1	1.8%
Ceftriaxone (25 µg)	52	92.9%

Antimicrobial Resistance Profile of *E. coli* isolated from Chickens in the Study Area: The *E. coli* isolates obtained during the study were highly resistant to the ten antibiotics tested (Table 2). All the isolates (100%) were resistant to Tetracycline, and the percentage of isolates resistant to others were Streptomycin (98.2%), Ceftriaxone (92.9%) and Sulphamethoxazole (85.7%), and Clindamycin (1.8%). (Table 2). All the isolates were resistant to at least three antimicrobials.

Discussion and Conclusion

Poultry production plays a significant role in ensuring food security in developing countries such as Nigeria, and contributes substantially to the availability of affordable animal protein. However, the increasing occurrence of antimicrobial-resistant (AMR) bacteria poses a serious challenge to poultry health, productivity and public health. Consequently, regular monitoring of antimicrobial resistance in poultry-associated bacteria is important.

Escherichia coli and *Enterococcus* species are among the earliest bacteria that colonize the intestinal tract of chickens, where they normally exist as commensals but may become opportunistic pathogens under favorable conditions. These organisms are useful indicators for monitoring AMR because they can readily acquire and transfer resistance determinants through genetic elements such as plasmids and transposons (Lipsitch and Samore, 2002). In the present study, the antimicrobial susceptibility of *E. coli* was examined as indicator of AMR in poultry farms within the Southeastern region of Nigeria. The isolates obtained in the present study demonstrated considerable resistance to several of the antimicrobials tested, suggesting a substantial reservoir of antimicrobial resistance within poultry populations in the study area. Such resistance may adversely affect poultry production and animal health and may also have important public health implications because of the close interaction between humans, poultry and the environment (Okoli *et al.*, 2002).

The detection of *E. coli* in 46.7% of the sampled chickens indicates a considerable level of intestinal colonization among the birds examined. This prevalence was higher than the 16.6% earlier reported in Owerri by Ugwu *et al*. (2022) and the 22.1% reported in Gusau by Abubakar *et al*. (2023); but it is lower than the 99.4% reported in Jos by Agusi *et al*. (2024). It is thought that the differences in prevalence across these locations may be associated with variations in farm management practices, environmental conditions, sanitation, stocking density and the health status of the birds. Although many *E. coli* strains are harmless commensals, some strains possess pathogenic characteristics and are collectively referred to as avian pathogenic *E. coli* (APEC). These organisms can cause diseases such as colibacillosis, particularly when birds are exposed to stressful conditions, poor sanitation, overcrowding, inadequate nutrition or compromised immunity. Diseases caused by pathogenic *E. coli* may manifest as respiratory, digestive or systemic disorders and can subsequently reduce poultry productivity.

Among the states investigated, Akwa Ibom State recorded the highest occurrence of *E. coli*, with 60.0% of the samples testing positive. Although this relatively high prevalence is notable, the present study did not establish a specific explanation for the observation. Further investigations are therefore required to determine whether farm management practices, environmental contamination, antimicrobial usage, biosecurity deficiencies, or other factors contributed to the higher occurrence in this State. Understanding these factors would be useful for developing appropriate control measures and reducing the circulation of potentially pathogenic and antimicrobial-resistant *E. coli* in poultry farms.

The antimicrobial susceptibility results obtained in this study showed that the isolated *E. coli* strains exhibited high levels of resistance to nine of the ten antimicrobial agents tested. Resistance to tetracycline, streptomycin, erythromycin and gentamicin was particularly pronounced. These findings are consistent with previous reports of antimicrobial resistance among *E. coli* isolates obtained from poultry. Hanchi *et al*. (2018), for example, reported that clinical *E. coli* isolates from chickens were resistant to tetracycline, chloramphenicol, ampicillin and aminoglycosides. Similar patterns have also been documented in countries such as Spain, Malaysia and the Netherlands (Blanco *et al*., 1997; Son and Gulam, 1995; VanDen Bogaard and Stobberingh, 1999). The consistently high resistance to commonly used antimicrobial agents across different geographical locations demonstrates the widespread nature of antimicrobial resistance in poultry-associated bacteria.

The extremely high resistance to tetracycline recorded in the present study may be associated with the extensive use of tetracycline in poultry production. Tetracyclines are commonly used for the prevention and treatment of bacterial infections in poultry and may also be incorporated into feed or drinking water. Repeated or inappropriate exposure to these agents has been reported to create selective pressure that favors the survival and multiplication of resistant bacterial strains. Similar high levels of resistance to tetracycline have previously been reported in Iran (Salehi and Bonab, 2006) and Malaysia (Geidam *et al*., 2012). Ugwu *et al*. (2015) also reported 100% tetracycline resistance among APEC strains isolated in Nsukka, Nigeria, which is comparable with the findings of the present study. These observations suggest that tetracycline resistance among poultry-associated *E. coli* has become an important concern in Nigeria.

The high level of resistance recorded in the present study may also be associated with the

increasing use of antibiotics as feed additives, for disease prevention and for therapeutic purposes. Inadequate regulation and inappropriate administration of antibiotics can contribute to the selection and persistence of resistant organisms. Furthermore, resistant *E. coli* may be introduced into poultry farms through contaminated water, feed, equipment, personnel, other animal species or the surrounding environment. Consequently, the antimicrobial resistance detected in the poultry sampled may not necessarily originate exclusively from antibiotic use within a particular farm. The movement of resistant bacteria between farms, animals, humans and environmental sources can contribute to the maintenance and dissemination of resistance within the wider community.

The genetic characteristics of *E. coli* further increase the concern associated with antimicrobial resistance. Resistance genes can be carried on mobile genetic elements such as plasmids and transposons, allowing them to move between bacterial cells. This horizontal transfer of resistance determinants can facilitate the development and dissemination of multidrug-resistant organisms (Liu *et al*., 2019; Mosquito *et al*., 2012). Some resistance genes encode enzymes capable of modifying or destroying antimicrobial compounds, thereby reducing or eliminating their effectiveness against the bacteria (Ramirez and Tolmasky, 2010). The ability of bacteria to acquire multiple resistance determinants therefore increases the possibility that infections caused by these organisms may become increasingly difficult to treat.

In the present study, resistance to more than one antimicrobial class was recorded among the *E. coli* isolates, indicating the occurrence of multidrug resistance. This finding agrees with the reports of Adenipekun *et al*. (2015) and Ejeh *et al*. (2017), which showed that resistance to multiple antimicrobial classes is characteristic of multidrug-resistant *E. coli* strains. The development of multidrug resistance may be linked to inappropriate, prolonged or repeated antimicrobial use in poultry production. The use of antibiotics for prophylaxis has particularly been associated with the selection and dissemination of resistant microorganisms (Okoli *et al*., 2006). Similarly, the continuous or improper use of the same antimicrobial agents in poultry farms can increase the likelihood that resistant bacterial populations will become established and persist over time (Manyi-Loh *et al*., 2018).

Despite the generally high resistance recorded in the present study for numerous antibiotics, the *E. coli* isolates demonstrated substantial susceptibility to clindamycin. One possible explanation is that clindamycin is not commonly established as an antimicrobial agent for routine poultry therapy in the study area. Its relatively limited use may therefore have resulted in lower selective pressure for resistance. Krishnamoorthy *et al*. (2017) similarly noted the potential usefulness of clindamycin in the treatment of certain *E. coli* infections.

Resistance to chloramphenicol and ciprofloxacin was comparatively similar in the present study, and was lower than what was previously reported in Imo State, Nigeria (Okoli *et al*., 2006). This finding is noteworthy because these antimicrobial agents are not relatively accessible within the study area. Conversely, the high level of resistance to gentamicin was unexpected because the drug is an older antimicrobial and is reportedly not extensively used in the study area. Gentamicin is generally administered by injection and is often administered with veterinary supervision, which could reduce routine exposure. Nevertheless, farmers may administer injectable antibiotics in severe cases, thereby creating opportunities for selection of resistant strains. Resistance may also be acquired through other environmental or genetic pathways, even where direct

exposure to a particular antimicrobial is limited (Kijima-Tanaka *et al*., 2003).

The antimicrobial resistance levels recorded in this study are comparable with previous findings involving coliform bacteria isolated from faecal and non-faecal samples (McKeon *et al*., 1995). The observed resistance may reflect the consequences of inappropriate antimicrobial use for prophylactic and therapeutic purposes in poultry production. The administration of antimicrobials at sub-therapeutic levels, prolonged use or inappropriate waste treatment practices can exert selective pressure on bacterial populations and promote the survival of resistant organisms. Furthermore, resistant *E. coli* originating from poultry may be transmitted to humans through direct contact, contaminated food, animal waste or environmental exposure. Nsofor and Iroegbu (2013) reported that both pathogenic and non-pathogenic antibiotic-resistant *E. coli* strains can potentially be transferred from chickens to humans through food.

The potential movement of antimicrobial resistance from food-producing animals to humans is of important public health concern. Human exposure to livestock and their faecal material, as well as the consumption and handling of animal-derived foods, provides opportunities for resistant bacteria and resistance genes to move between animal and human populations. The results of the present study indicate that chickens in the study areas harbor *E. coli* strains resistant to several antimicrobial agents that are commonly used in both veterinary and human medicine. Poor housing conditions, inadequate sanitation, high stocking densities and insufficient biosecurity may increase the occurrence and transmission of bacterial infections in poultry farms. This may subsequently increase farmers' reliance on antimicrobial agents for disease prevention and treatment, further contributing to the development of resistance.

Overall, the findings in the present study demonstrate that *E. coli* remains an important bacterium in poultry production because of its dual role as a normal intestinal organism and a potential opportunistic pathogen. Although many strains are harmless, pathogenic strains can reportedly cause respiratory infections, septicemia and other systemic diseases in poultry that compromise flock health and productivity. In addition, contaminated poultry and poultry products can represent a potential source of foodborne infection for humans. Effective control therefore requires a combination of good farm hygiene, appropriate sanitation, adequate nutrition, proper carcass handling, safe food preparation and effective biosecurity practices. Regular cleaning and disinfection of poultry facilities, monitoring of bird health, isolation of sick birds, proper assessment before movement of birds and poultry products and appropriate management practices can help reduce the spread of *E. coli* and other resistant organisms.

Finally, the high levels of antimicrobial resistance recorded in this study highlight the need for continuous surveillance of AMR among poultry-associated bacteria in Nigeria. Government agencies, researchers, veterinarians, poultry farmers and other relevant stakeholders should work together to promote responsible antimicrobial use and strengthen disease-prevention practices. Periodic monitoring can provide important information on changing resistance patterns and help guide appropriate antimicrobial selection and control strategies. Government policies supporting antimicrobial stewardship, improved veterinary oversight, farmer education, biosecurity and routine AMR surveillance are therefore essential for limiting the emergence and dissemination of resistant bacteria in poultry production and protecting both animal and human health (Chima *et al*., 2011).

Conflict of interest

No conflict of interest exists.

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