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High Frequency of Antibiotic-resistant *Enterobacterales* Contaminating Chicken Viscera in Nigeria: A Trigger for Foodborne Disease Outbreak

Ikechukwu Benjamin Moses[1], Emmanuel Nnabuike Ugbo[1], Aswin Rafif Khairullah[2], Wiwiek Tyasningsih[3], Mustofa Helmi Effendi[4], Chidimma Ruth Chukwunwejim[5], Ukpai Ekenem Grace[6]

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ABSTRACT

Background: Most of the *Enterobacterales* strains are non-pathogenic; however, there is increasing concern that some antibiotic-resistant strains of *Shigella* spp., *E. coli*, *Klebsiella* spp. and *Salmonella* spp. could spread from animals and their by-products to humans and cause different diseases. This study aimed to isolate, characterize and assess the antimicrobial resistance profiles of clinically important *Enterobacterales* isolated from chicken viscera samples in Nigeria.

Methods: A total of 200 chicken viscera (intestine, gizzard, liver and crop) samples obtained from various poultry slaughterhouse outlets were analyzed using standard microbiological procedures. Using the disc diffusion technique, the detected *Enterobacterales* isolates were tested for antibiotic susceptibility.

Result: Results showed that *Salmonella* spp., *E. coli*, *Klebsiella* spp. and *Shigella* spp. were members of the *Enterobacterales* recovered from the chicken viscera samples with frequency of 88 (44%), 72 (36%), 60 (30%) and 32 (16%) respectively. Additionally, the highest frequency of *Enterobacterales* isolation was from chicken intestine with a frequency of 92/252 (36.5%). Generally, all the recovered *Enterobacterales* were resistant (100%) to tetracycline and erythromycin, except for *Klebsiella* spp in which 16.7% resistance frequency was observed. Resistances were also observed with ampicillin (37.5%-100%), gentamicin (34.4%-66.7%) and ciprofloxacin (12.5%-83.3%). The average multiple antibiotic resistance index (MARI) value of the recovered isolates in this study was 0.7. This study has shown that chicken viscera parts are contaminated by multidrug-resistant (MDR) *Enterobacterales* such as *Shigella* spp., *E. coli*, *Klebsiella* spp. and *Salmonella* spp.

Key words: Antibiotics, Chicken viscera, *Enterobacterales*, Multidrug-resistant, Public health.

INTRODUCTION

Chicken meats, including those from laying hens and broiler chickens, are among the livestock products that are frequently eaten. Interestingly, poultry viscera parts are also consumed by humans in many developing countries (Castellano *et al.,* 2018; Effendi *et al.*, 2022; Hidayatik *et al.*, 2024), including Nigeria. When two or more people contract the same disease after consuming comparable food tainted with bacteria, it's known as a foodborne outbreak (Yanestria *et al.*, 2019). It has been determined to be one of the main reasons why people get sick and die. Foodborne illnesses are now a threat to and a barrier to global economic development, thus every nation spends a great deal of effort and money fighting them (Robertson *et al.*, 2018; Tyasningsih *et al.*, 2022).

Enterobacterales, a group of Gram-negative, facultative anaerobic and rod-shaped bacteria are well-known common inhabitants of the gastrointestinal tracts of humans, poultry and other animals. The majority of *Enterobacterales* strains are not harmful, but there is growing worry that certain strains of *Shigella* spp., *E. coli*, *Klebsiella* spp. and *Salmonella* spp., which are resistant to antibiotics, may transfer from animals and their waste products to humans and cause a variety of illnesses like

[1]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria.
[2]Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia.
[3]Division of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.
[4]Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.
[5]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.
[6]Department of Microbiology and Parasitology, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.

Corresponding Author: Mustofa Helmi Effendi, Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.
Email: mhelmieffendi@gmail.com

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hemolytic-uremic syndrome, enteritis, endocarditis, meningitis and urinary tract infections, all of which can have fatal consequences. (Rahman *et al.*, 2020; levy *et al.*, 2022). Among chicken, the *Enterobacterales* group has been linked to coli granuloma, cellulitis, omphalitis, yolk sac infection, swollen head syndrome and colibacillosis (Islam *et al.*, 2021; Gymoese *et al.,* 2017).

During the handling, transportation and slaughter procedures, equipment such contaminated tables, cutting supplies and packaging can cause cross-contamination of chicken products (Fagbamila *et al.*, 2017). Poor hygiene standards are the primary cause of the rising burden of foodborne illnesses, particularly in Africa. Microbial pathogens that cause foodborne illnesses may be acquired by food handlers through poor personal hygiene and inappropriate handling of meat products in abattoirs (Kusnoto *et al.*, 2024). In poultry farming, antibiotics are frequently utilized as prophylactics, treatments, or growth promoters (Roy *et al.*, 2022). The overuse and misuse of antibiotics is a major factor in the creation and spread of antibiotic-resistant *Enterobacterales*, which can infect people through food or direct contact with ill animals (Founou *et al.*, 2016; Wibisono *et al.*, 2021).

The evolution of antimicrobial resistance (AMR) is the most controversial topic affecting the health of people, animals and ecosystems in the twenty-first century (Urmi *et al.*, 2021; Yanestria *et al.*, 2022). Antimicrobial resistance constitutes one of the greatest challenges in modern medicine as it compromises and limits therapeutic options for the treatment of bacterial infections (Riwu *et al.*, 2022; Hemamalini *et al.*, 2025). Various mechanisms can lead to antimicrobial resistance in *Enterobacterales*, such as drug-expelling active efflux pumps, decreased cellular permeability, increased production of enzymes that modify or inactivate medicines and target mutations that result in modification (Ranjbar and Farahani, 2019). Investigating and understanding the contamination of poultry products by antibiotic-resistant *Enterobacterales* contributes to enhancing food safety as this helps in implementing measures to minimize the risk of foodborne illnesses associated with the consumption of contaminated poultry (Putri *et al.*, 2023).

Although, there are some reports on bacterial contamination of food-producing animals and their by-products; it is imperative to keep track of the current spread of antibiotic-resistant bacterial pathogens in order to develop strong preventing measures that will curtail AMR spread in both veterinary and human medicine. Additionally, there is still paucity of publicly available information on the public health implications of using poultry viscera as human food without proper processing, especially in developing countries, including Nigeria. This study, was therefore designed to isolate, characterize and determine the frequency of antibiotic-resistant *Enterobacterales* contaminating chicken viscera sold in different poultry slaughterhouse outlets in Abakaliki, Southeastern Nigeria.

MATERIALS AND METHODS

Collection of samples

A total of 200 chicken viscera samples of intestine (50), gizzard (50), liver (50) and crop (50) were aseptically collected from broiler chickens in various poultry slaughterhouse outlets between July and November, 2023. All aseptically collected chicken viscera parts were firstly cut open with small sterile scalpel blade and thereafter, swabs of the incised viscera parts were taken using sterile cotton swab sticks. Collected swab samples were labelled and placed in sample bags and then transported on ice packs within one hour of collection to the Department of Applied Microbiology laboratory, Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis.

Processing of samples and bacteriological analysis

Prior to being incubated for 18 to 24 hours at 37°C, collected swabs were first enriched in buffered peptone water (Oxoid, Hampshire, UK). Following the transfer of a loop full of the inoculated peptone water onto Salmonella-Shigella (SS) agar (used to isolate *Salmonella* spp. and *Shigella* spp.), MacConkey agar (used to isolate *Klebsiella* spp.) and Eosine Methylene Blue (EMB) agar (used to isolate *E. coli*) plates, the plates were incubated for 24 hours at 37°C. All culture media were purchased from Oxoid, Hampshire, UK. Colonies typical of *Salmonella* spp. (colourless colonies with dark centre on SS agar), *Shigella* spp. (smooth colourless colonies on SS agar), *E. coli* (greenish-metallic sheen on EMB agar) and *Klebsiella* spp. (mucoid pink-coloured colonies on MacConkey agar) were respectively sub-cultured through successive streaking in order to obtain pure colonies which were then subjected to further physiological and biochemical characterization such as Gram-staining, catalase, indole, oxidase, methyl red, citrate utilization, urease, Voges-Proskauer and nitrate reduction tests (Cheesbrough, 2006; Moses *et al.*, 2018; Pradika *et al.*, 2019; Widodo *et al.*, 2022). Pure colonies of identified isolates were then preserved and stored on nutrient agar slant at 4°C for further tests.

Antimicrobial susceptibility testing

The Clinical and Laboratory Standards Institute's (CLSI, 2022) criteria for the disc diffusion method on Mueller-Hinton agar were followed in order to determine the antimicrobial susceptibility tests. Mueller-Hinton (MH) agar (Oxoid, UK) was produced in compliance with the guidelines provided by the manufacturer. The medium was cooled, poured into plates and allowed to solidify before use. The test isolate's broth culture, which was 18-24 hours old, was standardized to the 0.5 McFarland standard. The standardized inoculums were placed inside a sterile swab stick, which was then drained to remove any extra inoculum load. The inoculums were then spread out onto a Mueller-Hinton agar plate surface. The Mueller-Hinton agar plate that had been inoculated was closed and left to dry at room temperature for a short while. The following antimicrobials

(Oxoid, UK) were tested against the test isolates: ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), ampicillin (10 µg) and tetracycline (30 µg). Using sterile forceps, all antibiotic discs (Oxoid, UK) were meticulously placed on the Mueller-Hinton agar plates that had been infected. Following the inoculation, the MH agar plates were incubated for 18 to 24 hours at 37°C in an aerobic environment. After incubation, inhibition zone diameters were measured, recorded and categorized as resistant, intermediate, or susceptible using the CLSI standards (CLSI, 2022). In this investigation, isolates with intermediate resistance were categorized as "resistant".

Determination of multiple antibiotic resistance index (MARI)

Each isolate's MARI value was determined using the disk diffusion method's interpreted findings. MARI was computed by dividing the total number of antibiotics tested against the isolate (y) by the number of antibiotics to which the isolate shows resistance (x). It is expressed as MARI = x/y (Moses *et al.*, 2020; Moses *et al.*, 2022).

Statistical analysis

The statistical evaluation of categorical variables was done with the SPSS version 17.0 statistical software package (SPSS Inc., Chicago, USA) using one-way between subjects ANOVA tool. The p-value of less than 0.05 (pvalue < 0.05) was deemed statistically significant for the results.

RESULTS AND DISCUSSION

Results showed that *Salmonella* spp. (Fig 1), *E. coli* (Fig 2), *Klebsiella* spp. (Fig 3) and *Shigella* spp. (Fig 4) were members of the *Enterobacterales* recovered from the 200 chicken viscera samples with frequencies of 88 (44%), 72 (36%), 60 (30%) and 32 (16%) respectively (Table 1). Additionally, the highest frequency of *Enterobacterales* isolation was from chicken intestines with a frequency of 92/252 (36.5%). This was closely followed by the crop 77/252 (30.6%), liver 43/252 (17.1%) and gizzard 40/252 (15.9%) (Table 1).

A one-way between subjects ANOVA was conducted to determine if the isolation frequency of *Enterobacterales* recovery is dependent on the type and nature of chicken viscera parts. Statistical analysis results showed that there was no statistically significant difference in the frequency of recovered *Enterobacterales* between the crop, gizzard, intestine and liver parts of chicken viscera that were analyzed in this study [F (3, 12) = 1.142, p = 0.372].

A one-way between subjects ANOVA was also conducted to determine if there is a statistically significant difference in the frequency of each major strains of *Enterobacterales* recovered from chicken viscera. Results also showed that there was no statistically significant difference in the frequency of recovered *E. coli*, *Klebsiella* spp., *Shigella* spp. and *Salmonella* spp from the viscera parts of chicken [F (3, 12) = 0.934, p = 0.454].

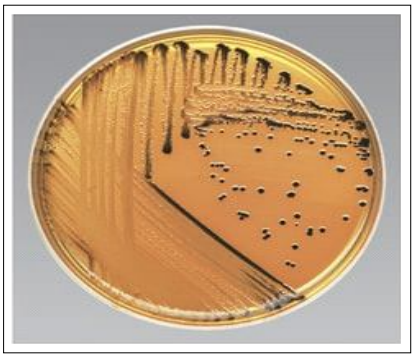

Fig 1: *Salmonella* spp. (colourless colonies with dark centre) on SS agar.

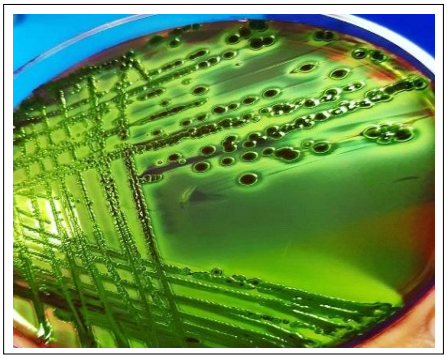

Fig 2: *E. coli* (greenish-metallic sheen colonies) on EMB agar.

Fig 3: *Klebsiella* spp. (mucoid pink-coloured colonies) on MacConkey agar.

Fig 4: *Shigella* spp. (smooth opaque colourless colonies) on SS agar.

Generally, all the recovered *Enterobacterales* (*Salmonella* spp., *E. coli*, *Klebsiella* spp. and *Shigella* spp.) were resistant (100%) to tetracycline and erythromycin, except for *Klebsiella* spp in which 16.7% resistance frequency was observed (Table 2). Resistances were also observed with ampicillin [(*Shigella* spp, 100%; *E. coli*, 37.5% and *Salmonella* spp., 37.5%)], gentamicin [(*Klebsiella* spp, 66.7%; *Shigella* spp., 34.4%)] and ciprofloxacin [(*Klebsiella* spp, 83.3%; *Shigella* spp, 34.4%; *E. coli*, 12.5% and *Salmonella* spp., 12.5%)]. The multiple antibiotic resistance index (MARI) value of the recovered isolates in this study ranged from 0.64-0.75 (Table 2).

A one-way between subjects ANOVA was also conducted to determine if there is a statistically significant difference in the resistance frequency of each major strains of *Enterobacterales* recovered from chicken viscera. From the results, no statistically significant difference was observed when the mean resistance frequencies of the recovered *E. coli*, *Klebsiella* spp., *Shigella* spp. and *Salmonella* spp. were compared [F (3, 16) = 0.340, p = 0.797].

Members of the *Enterobacterales* group such as *E. coli*, *Klebsiella* spp., *Salmonella* spp. and *Shigella* spp. are ubiquitous in nature and have been recognized as common inhabitants of the intestinal tracts of food-producing animals. Importantly, they are well-known foodborne pathogens associated with foodborne illnesses in humans and also the cause of mortality in commercial poultry and other food-producing animals (Ewers *et al.*, 2004; Pradika *et al.*, 2019; Widodo *et al.*, 2022).

In this study, different strains of the *Enterobacterales* group, especially *Salmonella* spp., *E. coli*, *Klebsiella* spp. and *Shigella* spp. were recovered from 200 chicken viscera samples with isolation frequency of 88 (44%), 72 (36%), 60 (30%) and 32 (16%) respectively. Interestingly, the frequency of *Enterobacterales* isolation was highest in chicken intestines with a frequency of 92/252 (36.5%). This was closely followed by the crop 77/252 (30.6%), liver 43/252 (17.1%) and gizzard 40/252 (15.9%). Additionally, all the recovered *Enterobacterales* (*Salmonella* spp., *E. coli*, *Klebsiella* spp. and *Shigella* spp.) were generally resistant (100%) to tetracycline and erythromycin, except for *Klebsiella* spp in which 16.7% resistance frequency was observed. Resistances (12.5%-100%) were also observed to ciprofloxacin, gentamicin and ampicillin. Isolates were multidrug-resistant with a mean multiple antibiotic resistance index (MARI) value of 0.7.

The frequency of *E. coli* (20.8%) recovered from liver in our study is higher than the 13.6% reported by Sarba *et al.* (2019). Interestingly, just like we reported *E. coli* prevalence in other chicken visceral parts such as gizzard (13.9%), crop (20.8%) and intestine (44.4%), Sarba *et al.* (2019) also reported *E. coli* in different chicken internal organs/parts such as the kidney (6.3%), spleen (15.2%) and ovary (10.7%). Dashe *et al.* (2003) also found that the isolation rate of *E. coli* was 15.8% from the liver and 13% from the spleen, indicating that *E. coli* is primarily found in these organs.

The frequency of *Klebsiella* spp, (30%) in our study also agrees with the study of Wareth and Neubauer (2021) who reported the isolation of *Klebsiella* spp. from poultry carcass and internal organs; although with a higher isolation frequency of 45%.

Shigella spp. was the least prevalent *Enterobacterales* recovered from chicken viscera samples analysed in our study with a frequency of 16%. Other studies have also reported *Shigella* spp. in animal products with frequency ranging from 1.4%-7% (Elkenany *et al.*, 2022; Ahmed and Shimamoto, 2014). Our study is also in concord with another study that reported *Salmonella* spp. in poultry products (Gad *et al.*, 2018).

Table 1: Distribution of *Enterobacterales* isolated from different visceral parts of chicken.

Chicken viscera part	No of samples	*Enterobacterales* isolated				Total isolated
		E. coli	*Klebsiella* spp.	*Shigella* spp.	*Salmonella* spp.	
Crop	50	15	10	32	20	77 (30.6%)
Gizzard	50	10	20	0	10	40 (15.9%)
Intestine	50	32	30	0	30	92 (36.5%)
Liver	50	15	0	0	28	43 (17.1%)
Total	200	72 (36%)	60 (30%)	32 (16%)	88 (44%)	252

Table 2: Antimicrobial susceptibility profiles of *Enterobacterales* isolated from different visceral parts of chickens.

Antibiotic	*E. coli*		*Klebsiella* spp.		*Shigella* spp.		*Salmonella* spp.	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Gentamicin	0	100	66.7	33.3	34.4	65.6	0	100
Erythromycin	100	0	16.7	83.3	100	0	100	0
Ciprofloxacin	12.5	87.5	83.3	16.7	34.4	65.6	12.5	87.5
Tetracycline	100	0	100	0	100	0	100	0
Ampicillin	37.5	62.5	0	100	100	0	37.5	62.5
MARI value	0.64		0.68		0.73		0.75	

There was no statistically significant difference in the frequency of recovered *Enterobacterales* between the crop, gizzard, intestine and liver viscera parts of chicken that were analyzed in this study (p=0.372). Although more *Enterobacterales* pathogens were recovered from the chicken intestine and crop; the differences in the frequency of *Enterobacterales* isolation in the 4 chicken viscera parts that were analysed is likely due to chance and not because of the nature of the chicken viscera parts. Additionally, there was no statistically significant difference in the frequency of recovered *E. coli*, *Klebsiella* spp., *Shigella* spp. and *Salmonella* spp from the viscera parts of chicken (p = 0.454). The recovery frequency of the *Enterobacterales* strains in this study is also likely due to chance.

The differences in isolation frequency of *Enterobacterales* in our study when compared with other studies might likely be due to differences in sample size, hygienic practices of the poultry product vendors and farmers, the sampling time/period and location. Possible sources of additional contamination of the chicken viscera might be the chicken itself, or from contamination during the processing (such as dressing, packaging, storage etc.), as good aseptic conditions were ensured from sample collection point to laboratory analysis during this study.

Antibiotic-resistant strains of the *Enterobacterales* group have also been reported to be common bacterial contaminants in retail poultry meat and other animal products (Anderson *et al.*, 2019) with significant public health threat and economic burden. Antibiotic-resistant bacterial pathogens such as *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) have also been implicated in the contamination of other animal products, especially cow milk in Indonesia (Pradika *et al.*, 2019; Widodo *et al.*, 2022; Khairullah *et al.*, 2022). A minimum of three distinct types of antibiotics were resistant to the isolated *Enterobacterales* in this investigation, indicating their multidrug resistance. Importantly, the recovered *Enterobacterales* (*Salmonella* spp., *E. coli*, *Klebsiella* spp. and *Shigella* spp.) exhibited resistance (12.5%-100%) to ciprofloxacin, gentamicin, ampicillin, erythromycin and tetracycline. Additionally, the average multiple antibiotics resistance index (MARI) value of the *Enterobactaerales* strains in this study ranged from 0.64-0.75 with a mean MARI value of 0.7; thus, depicting the misuse of antibiotics in our study area. The antimicrobial susceptibility testing results in this study showed varying patterns of responses to tested antibiotics by the different strains of the *Enterobacterales* group. Interestingly, some antimicrobials (especially ampicillin and gentamicin) that were completely active (100%) against all the recovered *E. coli* and *Salmonella* spp. isolates were ineffective against some other *Enterobacterales* strains of *Klebsiella* spp. and *Shigella* spp. which exhibited resistance frequencies of 66.7% and 34.4% respectively.

Similar patterns of antimicrobial resistance, including multidrug-resistant (MDR) strains that were observed in our study have also been reported in other studies. Sarba *et al.* (2019) reported high frequency of MDR *E. coli* recovered from chicken internal organs. In their study, resistances were noted for fluoroquinolones (100%), aminoglycosides (69.9-89%), beta-lactams (84.6%) and macrolides (75%). Our study also agrees with the work of Adenipekun *et al.* (2015) who also isolated MDR *E. coli* in chicken products. Anderson, (2019) reported that all the *Shigella* spp. isolates recovered from chicken products were resistant to beta-lactam antibiotics. Okoli *et al.* (2021) also reported that all the *Salmonella* and *Shigella* isolates recovered from chickens in their study were resistant to the β-lactam antibiotics. MDR *Enterobacterales* recovered from chicken products with similar antibiotic resistance patterns to the isolates in our study have also been reported in by other authors (Gu *et al.*, 2015; Teimourpour *et al.*, 2019; Ben *et al.,* 2019; Elkenany *et al.*, 2022; Shoja *et al.*, 2023).

No statistically significant difference was observed when the mean resistance frequencies of the recovered *Salmonella* spp, *Klebsiella* spp., *E. coli* and *Shigella* spp. in this study were compared (p=0.797). Their different resistance frequency is likely due to chance. Results of this study further emphasizes the importance of conducting antimicrobial susceptibility testing, especially in cases of foodborne illnesses/diseases, in order to determine the most effective therapeutic antimicrobial options and also to curtail the increasing spread of antimicrobial resistance in both human and veterinary medicine which might result from wrong/indiscriminate antimicrobial prescription/usage (Yanestria *et al.*, 2024).

The widespread occurrence of antimicrobial resistance within or between antibiotic classes in different studies may be the result of incorrect or careless antimicrobial usage in human, veterinary and agricultural medicine (Khairullah *et al.*, 2024). Similar antibiotics are often used indiscriminately over extended periods of time, particularly in poultry farms, which may encourage the formation and spread of bacteria resistant to antibiotics (Riwu *et al.*, 2024). This is why, today, antimicrobial resistance has become a serious growing global public health problem in both veterinary and human medicine (Li *et al.*, 2021).

To reduce the risk of contamination by MDR *Enterobaterales*, improved hygiene and sanitation practices during poultry production and processing should be implemented. This may include improved sanitation of poultry housing and slaughtering facilities, as well as better hygiene practices by poultry workers. These measures could help reduce the risk of foodborne disease outbreak.

CONCLUSION

This study has shown that chicken viscera parts sold in poultry slaughterhouse outlets in Abakaliki, Nigeria are highly contaminated with multidrug-resistant (MDR) *Enterobaterales*. Among the recovered *Enterobacterales*, *Salmonella* spp. was the most prevalent (44%), followed

by *E. coli* (36%), *Klebsiella* spp. (30%) and *Shigella* spp. (16%). Interestingly, the highest frequency of *Enterobacterales* isolation was from chicken intestines 92/252 (36.5%). Generally, all the recovered *Enterobacterales* were resistant (100%) to tetracycline and erythromycin, except for *Klebsiella* spp in which 16.7% resistance frequency was observed. Resistance was also observed with ampicillin (37.5%-100%), gentamicin (34.4%-66.7%) and ciprofloxacin (12.5%-83.3%). The detection of MDR *Enterobacterales* in chicken viscera as observed in our study calls for strong measures that would safeguard human health and also curb the increasing incidence and spread of superbugs in the food chain which could be accompanied by serious public health consequences, especially foodborne disease outbreaks. Additionally, public health advocacy such as sensitization of poultry farmers on good hygienic practices, prudent use of antibiotics and the continuous monitoring of poultry farms and other outlets where poultry products are sold to the public for MDR strains of *Enterobacterales* are very vital in the containment of foodborne diseases.

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Conflict of interest

All authors declare that they have no conflict of interest.

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