

Ojja et al. Afr. J. Clin. Exper. Microbiol. 2024; 25 (2): 210 – 218

<https://www.africem.org>African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X  
AJCEM/2305. <https://www.ajol.info/index.php/ajcem>

Apr 2024; Vol.25 No.2

Copyright AJCEM 2024: <https://dx.doi.org/10.4314/ajcem.v25i2.12>**Original Article****Open Access**

## **Antimicrobial resistance profiles of bacteria from Enterobacteriaceae family of laying chicken in Ibadan, southwestern Nigeria**

\*<sup>1</sup>Ojja, C. V., <sup>2</sup>Amosun, E. A., and <sup>3</sup>Ochi, E. B.

<sup>1,2</sup>Avian Medicine Programme, Pan African University Life and Earth Sciences Institute (Including Health and Agriculture), University of Ibadan, Ibadan, Nigeria

<sup>2</sup>Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

<sup>3</sup>Department of Clinical Studies, School of Veterinary Medicine, University of Juba, Juba, South Sudan

\*Correspondence to: [ojja.christopher@paulesi.org.ng](mailto:ojja.christopher@paulesi.org.ng); [vukenichris170@gmail.com](mailto:vukenichris170@gmail.com); +211923419563

**Abstract:**

**Background:** Antibiotics are significant for improving the health and productivity of chickens, but overuse and misuse of antibiotics have led to the development of antimicrobial resistance (AMR), which has resulted in ineffective treatment of infectious diseases with associated mortality in chicken and potential spread of AMR pathogens to humans. The objective of the study was to evaluate the AMR profiles of *Enterobacteriaceae* from faecal samples of laying chicken in Ibadan, southwestern Nigeria.

**Methodology:** This was a cross-sectional study of 200 apparently healthy laying hens from 10 selected local government areas of Ibadan, Nigeria, and from which cloacal samples were collected for isolation of *Enterobacteriaceae*. Samples were first inoculated on tryptone soy broth (TSB) for enrichment and then subcultured on MacConkey agar plates. Presumptive *Escherichia coli* isolates were sub-cultured on Eosin Methylene Blue (EMB) agar and greenish metallic sheen colonies on EMB agar were identified as *E. coli* by colony morphology and Gram stain microscopy. Commercial API (Analytical Profile Index) kit was used to confirm the identity of the *Enterobacteriaceae* isolates. Antimicrobial susceptibility testing of the isolates was performed by the disc diffusion technique and result interpreted using the guideline of Clinical and Laboratory Standards Institute. Data were analysed on STATA and  $p < 0.05$  was considered statistical significance.

**Results:** The results showed that out of 200 chicken samples, 190 were cultured positive, giving a colonization rate of 95.0%, with 287 *Enterobacteriaceae* isolates. *Escherichia coli* (59.6%), *Enterobacter* spp., (27.9%), and *Klebsiella pneumoniae* (12.5%) were the bacterial isolates identified. For antibiotic susceptibility, *E. coli* had sensitivity rate of 78.2% to ciprofloxacin, 73.4% to ofloxacin, 71.8% to sparfloxacin, and 70.9% to pefloxacin, and resistant rates to cotrimoxazole of 73.4%, streptomycin 65.4%, and other antibiotics 63.7%. *Klebsiella pneumoniae* was sensitive to gentamicin (33.3%), ofloxacin (33.3%), and ciprofloxacin, but resistant to other antibiotics. *Enterobacter* spp. was sensitive to amoxicillin-clavulanic acid (93.8%), pefloxacin, and streptomycin (70.3%), but resistant to ofloxacin (100.0%), cotrimoxazole (84.5%), chloramphenicol (68.8%), gentamicin (64.1%), amoxicillin (60.9%) and ciprofloxacin (60.9%). A total of 29 resistance patterns were observed in 50 resistant *Enterobacteriaceae* isolates with 12 MDR patterns observed in 54.0% ( $n=27$ ) of the isolates.

**Conclusion:** This study reports faecal *Enterobacteriaceae* colonization rate of 95% of commercial poultry chicken in Ibadan, southwest Nigeria, belonging to three members of the family *Enterobacteriaceae*, with high MDR patterns. The high AMR rates can lead to ineffective treatment of infectious diseases in chicken, with associated mortality and a potential source for transmission of AMR pathogens to humans.

**Keywords:** Antimicrobial resistance; *Enterobacteriaceae*; Laying chicken; Ibadan; Nigeria

Received Oct 17, 2023; Revised Jan 25, 2024; Accepted Jan 27, 2024

Copyright 2024 AJCEM Open Access. This article is licensed and distributed under the terms of the Creative Commons Attribution 4.0 International License <http://creativecommons.org/licenses/by/4.0/>, which permits unrestricted use, distribution and reproduction in any medium, provided credit is given to the original author(s) and the source. Editor-in-Chief: Prof. S. S. Taiwo

## **Profils de résistance aux antimicrobiens des bactéries de la famille des Enterobacteriaceae des poules pondeuses à Ibadan, dans le sud-ouest du Nigeria**

\*<sup>1</sup>Ojja, C. V., <sup>2</sup>Amosun, E. A., et <sup>3</sup>Ochi, E. B.

<sup>1,2</sup>Programme de Médecine Aviaire, Institut des Sciences de la Vie et de la Terre de l'Université Panafricaine (y Compris la Santé et l'Agriculture), Université d'Ibadan, Ibadan, Nigéria

<sup>2</sup>Département de Microbiologie et de Parasitologie, Faculté de Médecine Vétérinaire, Université d'Ibadan, Ibadan, Nigéria

<sup>3</sup>Département d'Etudes Cliniques, École de Médecine Vétérinaire, Université de Juba, Juba, Soudan du Sud

\*Correspondance à: [ojja.christopher@paulesi.org.ng](mailto:ojja.christopher@paulesi.org.ng); [vukennichris170@gmail.com](mailto:vukennichris170@gmail.com); +211923419563

## Résumé:

**Contexte:** Les antibiotiques sont importants pour améliorer la santé et la productivité des poulets, mais leur utilisation excessive et inappropriée a conduit au développement d'une résistance aux antimicrobiens (RAM), qui a entraîné un traitement inefficace des maladies infectieuses avec une mortalité associée chez les poulets et une propagation potentielle de ces maladies. Agents pathogènes de la RAM pour les humains. L'objectif de l'étude était d'évaluer les profils de RAM des *Entérobactéries* à partir d'échantillons fécaux de poules pondeuses à Ibadan, dans le sud-ouest du Nigeria.

**Méthodologie:** Il s'agissait d'une étude transversale portant sur 200 poules pondeuses apparemment en bonne santé provenant de 10 zones de gouvernement local sélectionnées d'Ibadan, au Nigeria, et à partir desquelles des échantillons cloacaux ont été collectés pour l'isolement des *Entérobactéries*. Les échantillons ont d'abord été inoculés sur un bouillon tryptone soja (TSB) pour enrichissement, puis repiqués sur des plaques de gélose MacConkey. Des isolats présumés d'*Escherichia coli* ont été sous-cultivés sur une gélose à l'éosine et au bleu de méthylène (EMB) et des colonies à reflets métalliques verdâtres sur une gélose EMB ont été identifiées comme étant *E. coli* par la morphologie des colonies et la microscopie à coloration de Gram. Un kit commercial API (Analytical Profile Index) a été utilisé pour confirmer l'identité des isolats d'entérobactéries. Les tests de sensibilité aux antimicrobiens des isolats ont été réalisés par la technique de diffusion sur disque et les résultats ont été interprétés selon les directives d'Institut des Normes Cliniques et de Laboratoire. Les données ont été analysées sur STATA et  $p < 0,05$  a été considéré comme statistiquement significatif.

**Résultats:** Les résultats ont montré que sur 200 échantillons de poulets, 190 étaient cultivés positifs, soit un taux de colonisation de 95,0%, avec 287 isolats d'*Entérobactéries*. *Escherichia coli* (59,6%), *Enterobacter* spp. (27,9%) et *Klebsiella pneumoniae* (12,5%) étaient les isolats bactériens identifiés. Pour la sensibilité aux antibiotiques, *E. coli* avait un taux de sensibilité de 78,2% à la ciprofloxacine, de 73,4% à l'ofloxacine, de 71,8% à la sparfloxacine et de 70,9% à la pefloxacine, et des taux de résistance au cotrimoxazole de 73,4%, à la streptomycine de 65,4% et à d'autres antibiotiques de 63,7%. *Klebsiella pneumoniae* était sensible à la gentamicine (33,3%), à l'ofloxacine (33,3%) et à la ciprofloxacine, mais résistante à d'autres antibiotiques. *Enterobacter* spp. était sensible à l'amoxicilline-acide clavulanique (93,8%), à la pefloxacine et à la streptomycine (70,3%), mais résistant à l'ofloxacine (100,0%), au cotrimoxazole (84,5%), au chloramphénicol (68,8%), à la gentamicine (64,1%), à l'amoxicilline (60,9 %) et ciprofloxacine (60,9%). Au total, 29 profils de résistance ont été observés dans 50 isolats d'*Enterobacteriaceae* résistants, avec 12 profils MDR observés dans 54,0% ( $n=27$ ) des isolats.

**Conclusion:** Cette étude rapporte un taux de colonisation fécale des *Enterobacteriaceae* de 95,0% des volailles commerciales d'Ibadan, dans le sud-ouest du Nigeria, appartenant à trois membres de la famille des *Enterobacteriaceae*, avec des profils MDR élevés. Les taux élevés de RAM peuvent conduire à un traitement inefficace des maladies infectieuses chez le poulet, avec une mortalité associée et une source potentielle de transmission d'agents pathogènes RAM aux humains.

**Mots-clés:** Résistance aux antimicrobiens; *Entérobactéries*; Poulet pondeur; Ibadan; Nigeria

## Introduction:

The *Enterobacteriaceae* is a broad family of Gram-negative bacteria that comprise both free-living and indigenous flora of the lower gastrointestinal tract of various animals and humans (1,2). The family comprises 51 genera and 238 species (3–5). Most of their members live in the intestines of chickens and humans, and have been widely studied due to their obvious impact on human and animal health as well as agricultural practices (5,6). The important members of the *Enterobacteriaceae* family that cause food poisoning, gastroenteritis, enteric fever, and plague include *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Citrobacter* spp., *Yersinia* spp., *Klebsiella* spp., and *Escherichia coli* (3).

Antimicrobials are important in improving chicken health and productivity in poultry, cattle, and aquaculture (7–9). However, excessive use of antimicrobials has envi-

ronmental and health implications due to the creation of antimicrobial resistance (AMR), leading to ineffective treatment of infectious diseases and death of animals and humans (10,11). In most rural and peri-urban areas of Nigeria and other developing countries, antimicrobials are readily purchased for use in chickens and humans without prescriptions, resulting in misuse, which has negatively affected their efficacy (3,12).

In a global context, antimicrobials such as antibiotics are widely used, at non-therapeutic dosages, as growth promoters, and prophylaxis in chicken feeds, and water, which sometimes last for long period, thereby predisposing to emergence of AMR strains of microorganisms (13). Also, when antibiotics are excreted in chicken wastes, they can contaminate the environment, thereby facilitating the widespread dissemination of AMR traits in the community (7–9,14).

The objective of this study is to det-

etermine the AMR profiles of *Enterobacteriaceae* isolates of faecal samples of laying chicken in Ibadan, southwestern Nigeria, with the aim of bridging significant knowledge gaps regarding the prevalence of this problem for urgent public health responses as well develop recommendations for farmers on the appropriate ways to apply antimicrobial medications to increase poultry productivity, improve food security and prevent emergence of AMR pathogens.

## **Materials and method:**

### **Study area:**

The study was conducted in Ibadan, a city in the southwestern region of Nigeria, at coordinates of latitude 7° 22' 36.2496" N and longitude 3° 56' 23.2296" E (Fig 1). Ibadan is located at an elevation of 230 meters above sea level and is divided into several LGAs (15,16).

### **Study design, participants and sample collection:**

This was a descriptive cross-sectional study of 200 apparently healthy chickens randomly selected from 10 poultry farms (20 per farm) in 10 LGAs (1 farm per LGA) in Ibadan, southwestern Nigeria between January 1 and 31, 2023 (Table 1). Chicken that exhibited symptoms and/or signs of sickness were excluded from the study.

Cloacal faecal samples were collected using sterile swab sticks, placed in cold box with ice packs, and transported to the laboratory of Veterinary Microbiology Department of the University of Ibadan for microbiological analysis.

### **Isolation and identification of *Enterobacteriaceae* isolates:**

Each sample was pre-enriched by inoculating cloacae faecal swab into 9ml sterile tryptone soy broth (TSB) in universal bottles, and incubated at 37°C for 18-24hrs. A loopful of the pre-enrichment culture was inoculated on MacConkey agar and incubated at 37°C for 18-24hours. Rose pink colonies from MacConkey agar were sub-cultured onto Eosin Methylene Blue agar (EMB) and incubated at 37°C for 24 hrs.

The rose-pink colonies on MacConkey agar plates (putative *E. coli*) that showed

greenish metallic sheen colonies on EMB agar were selected for phenotypic identification, which included observation of colony morphology, Gram stain microscopy reaction, and commercial biochemical identification with Analytical Profile Index (API) to confirm the identity of *E. coli* and other bacteria isolates.

### **Antibiotic susceptibility testing (AST):**

Antimicrobial susceptibility testing of the isolates was performed by the disc diffusion technique recommended by the Clinical and Laboratory Standards Institute (17). Five classes of antibiotics were tested which included fluoroquinolones (ofloxacin, ciprofloxacin, pefloxacin, sparfloxacin); sulfonamide (co-trimoxazole); phenicol (chloramphenicol); penicillins (amoxicillin, amoxicillin-clavulanic acid); and aminoglycoside (gentamicin, streptomycin). The antibiotic discs used were amoxicillin (AM, 30µg); amoxicillin-clavulanic acid (augmentin or AU 10µg); ofloxacin (tarivid or OFX 10µg); sparfloxacin (SP 10µg); ciprofloxacin (CPX 30µg); pefloxacin (PEF 30µg); chloramphenicol (CH 30µg); sulfamethoxazole-trimethoprim (septrin or SXT 30µg); streptomycin (S 30µg) and gentamicin (CN 30 µg).

Inoculum suspension of the overnight colonies of each isolate was prepared and adjusted to match the turbidity of 0.5 McFarland standards. Mueller-Hinton (MH) agar was inoculated with the suspension using a sterile swab. A sterile forcep was used to place the antibiotic discs on the inoculated MH agar plate, and the seeded agar plates were incubated for 24 hours at 37°C. The measurement of the diameter of inhibition zones around the antibiotic discs was done to the nearest millimeter, and interpreted according to the guidelines of the Clinical and Laboratory Standards Institutes (17). Multi-drug resistance (MDR) in any isolate was defined as resistance to antibiotics in 3 or more of the 5 classes of antibiotics tested (18).

### **Data analysis:**

Data were entered into the Microsoft Excel 2010, including the samples checklist, and laboratory results, before sending them into STATA 17.0 version for analysis. All the results are presented in tables and graphs as frequencies, and percentages.

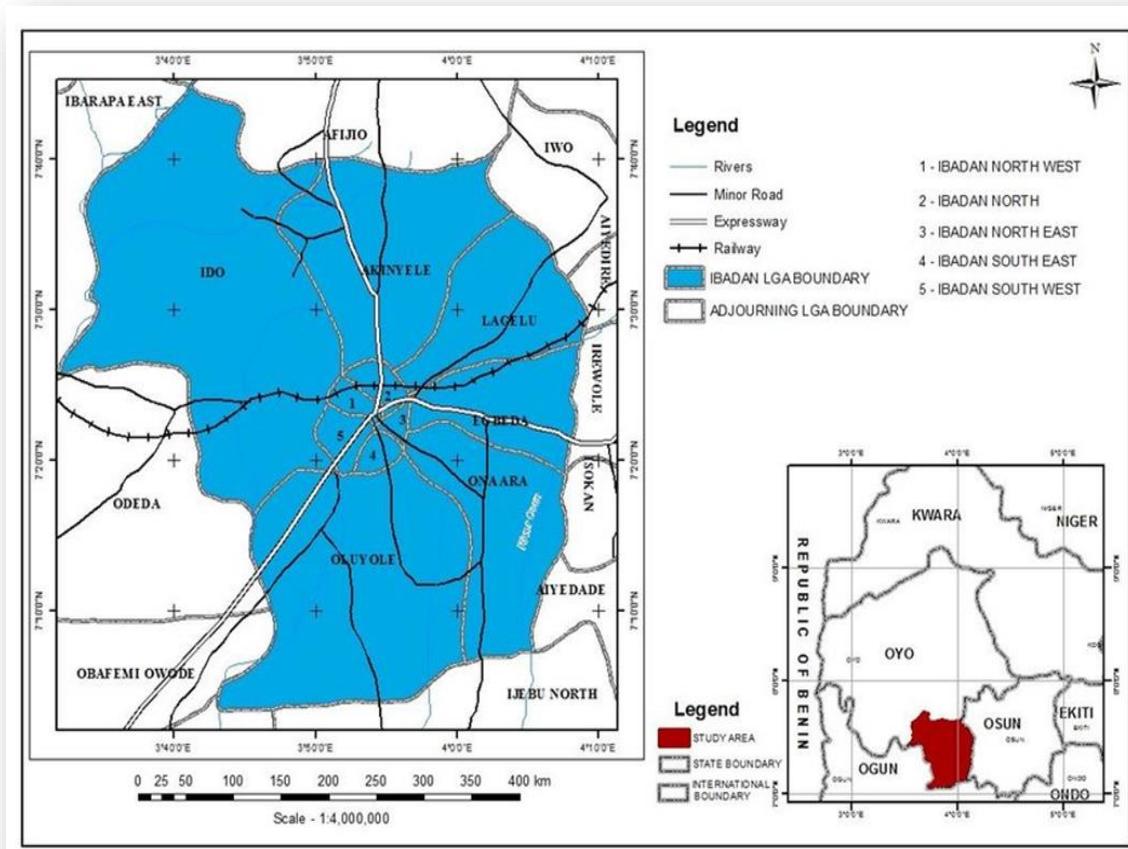


Fig 1: Map of the study area

Table 1: Distribution of laying chickens and sample collection with respect to the study location and local government areas (LGAs) in Ibadan, Nigeria

Local Government Areas	Farms	Poultry type	Samples collected	Number of chicken samples collected
Akinyele	Elyon	Laying Hens	Cloacal fecal	20
Ibadan North	BL Goshe	Laying Hens	Cloacal fecal	20
Lagelu	Olu Oluwa	Laying Hens	Cloacal fecal	20
Ibadan North East	Freedom	Laying Hens	Cloacal fecal	20
Ido	His Grace	Laying Hens	Cloacal fecal	20
Ibadan North West	James's	Laying Hens	Cloacal fecal	20
Ona Ara	Kay's	Laying Hens	Cloacal fecal	20
Egbeda	Ayo's	Laying Hens	Cloacal fecal	20
Ibadan South East	Twins'	Laying Hens	Cloacal fecal	20
Oluoyole	Sun Boy's	Laying Hens	Cloacal fecal	20

## Results:

### Prevalence of faecal colonization of chicken by Enterobacteriaceae:

Cloacal faecal samples of 190 chickens were culture positive for Enterobacteriaceae, giving a colonization rate of 95.0%

while 10 (5.0%) were culture negative. Three species of bacteria belonging to family Enterobacteriaceae were isolated, with *E. coli* in 121 chickens (60.5%), *Enterobacter* spp. in 46 chickens (23.0%), and *Klebsiella pneumoniae* in 23 chickens (11.5%) as shown in Fig 2.

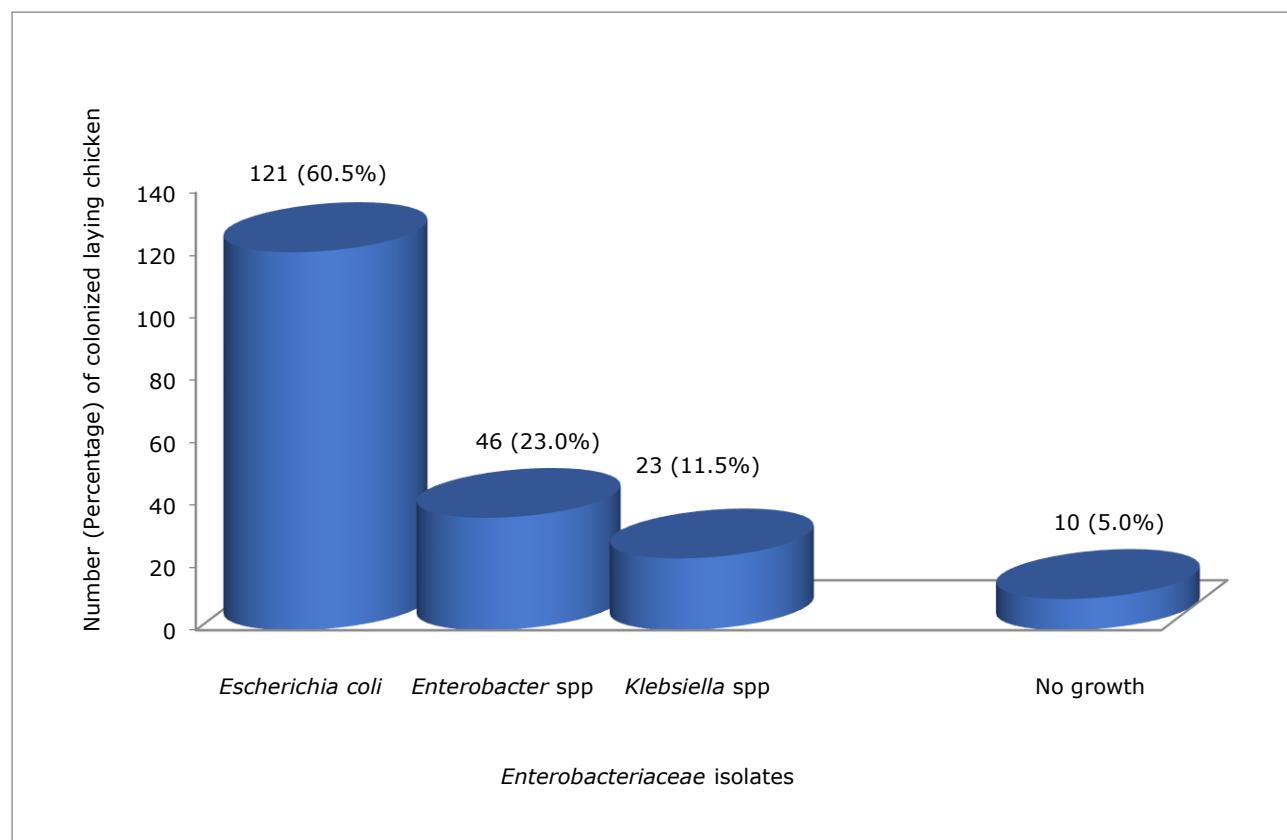
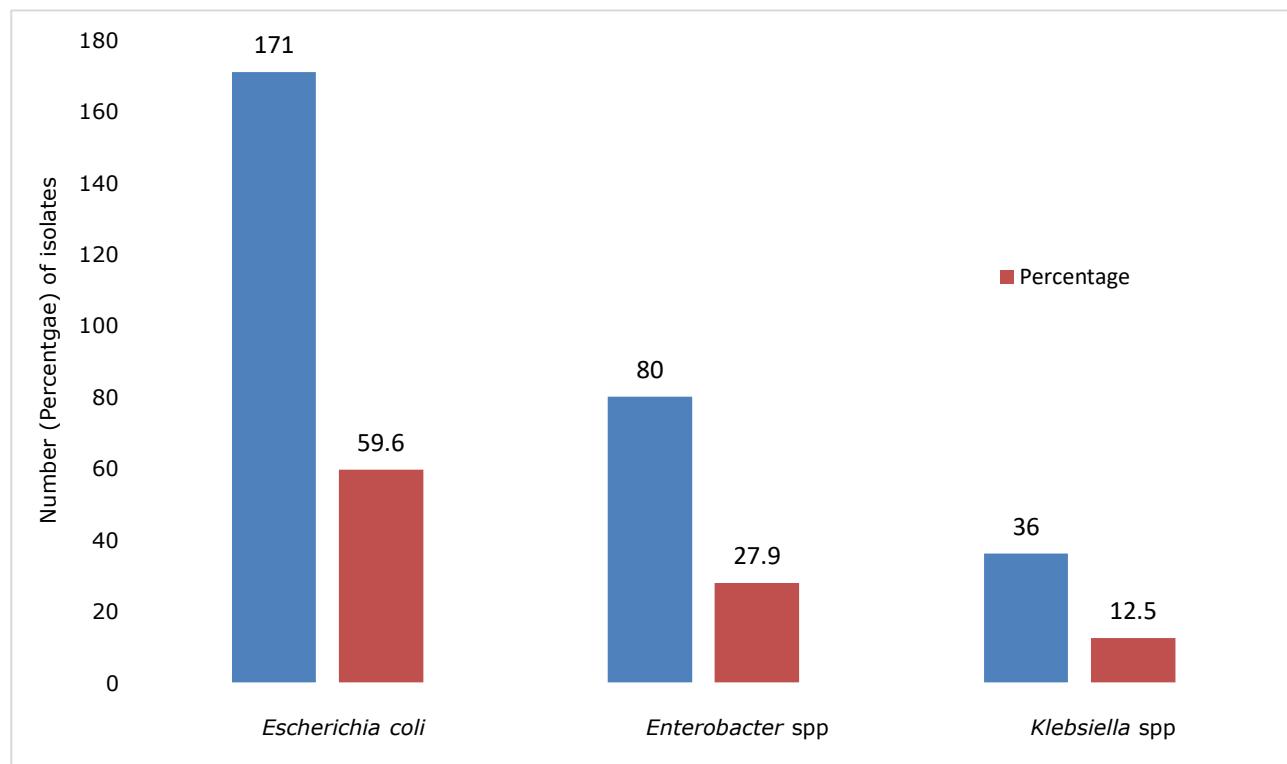
Fig 2: Prevalence of faecal colonization of laying chickens by *Enterobacteriaceae*

Fig 3: Frequency distribution of bacterial isolates from positive cultures

**Frequency distribution of the *Enterobacteriaceae* isolates:**

A total of 287 bacterial isolates were recovered from the 190 positive cultures, with the most frequent being *E. coli* (171, 59.6%), *Enterobacter* spp. (80, 27.9%), and *Klebsiella pneumoniae* (36, 12.5%) as shown

in Fig 3.

The farms with the highest number of isolates were Elyon and BL Goshe farm with 39 (13.6%) each, followed by Freedom farm with 33 (11.5%), and Sun's Boy farm with 19 (6.6%) ( $p>0.05$ ) as shown in Table 2.

Table 2: Frequency distribution of *Enterobacteriaceae* isolates with respect to farms and local government areas in Ibadan

Sample location/ farm	LGAs	No of chicken sampled	No of LF isolates	No of NLF isolates	Total no of isolates (%)	p value
Elyon	Akinyele	20	25	14	39 (13.6)	
BL Goshe	Ibadan North	20	19	20	39 (13.6)	
Olu Oluwa	Lagelu	20	21	09	30 (10.5)	
Freedom farm	Ibadan North East	20	17	16	33 (11.5)	
His Grace	Ido	20	11	12	23 (8.0)	
James's farm	Ibadan North West	20	15	07	22 (7.7)	
Kay's farm	Ona Ara	20	18	3	21 (7.3)	
Ayo's farm	Egbeda	20	24	8	32 (11.5)	
Twins	Ibadan South East	20	22	7	29 (10.1)	
Sun Boy's farm	Oloyole	20	11	8	19 (6.6)	
<b>Total</b>		<b>200</b>	<b>183</b>	<b>104</b>	<b>287 (100.0)</b>	$p>0.05$

LF: Lactose fermenter; NLF: Non-lactose fermenter; LGA: Local Government Area

Table 3: Antibiotic susceptibility of *Enterobacteriaceae* isolates from laying chicken in Ibadan, Nigeria

Antibiotic class	<i>Escherichia coli</i>		<i>Klebsiella pneumoniae</i>		<i>Enterobacter</i> spp	
	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)
<b>Penicillin</b>						
AM	171 (100.0)	0	12 (33.3)	24 (66.7)	25 (39.1)	39 (60.9)
AU	128 (75.0)	43 (25.0)	10 (27.8)	26 (72.2)	60 (93.8)	4 (6.2)
<b>Fluoroquinolones</b>						
OFX	118 (69.0)	53 (31.0)	19 (52.8)	17 (47.2)	0	64 (100)
SP	10 (6.0)	161 (94.0)	17 (47.2)	19 (52.8)	30 (46.9)	34 (53.1)
CPX	147 (86.0)	24 (14.0)	25 (69.4)	11 (30.6)	25 (39.1)	39 (60.9)
PEF	125 (73.0)	46 (27.0)	19 (52.8)	17 (47.2)	45 (70.3)	19 (29.7)
<b>Phenicol</b>						
CH	100 (58.0)	71 (42.0)	10 (27.8)	26 (72.2)	20 (31.3)	44 (68.8)
<b>Sulfonamide</b>						
SXT	50 (29.0)	121 (71.0)	8 (22.2)	28 (77.8)	10 (15.6)	54 (84.4)
<b>Aminoglycoside</b>						
S	48 (28.0)	123 (72.0)	12 (33.3)	24 (66.7)	45 (70.3)	19 (29.7)
CN	21 (12.0)	150 (88.0)	0	36 (100.0)	23 (35.9)	41 (64.1)

AM=amoxicillin; AU=amoxicillin-clavulanic acid (Augmentin); OFX=ofloxacin (Tarivid); SP=Sparfloxacin; CPX=ciprofloxacin; PEF=pefloxacin;  
CH=chloramphenicol; SXT=sulfamethoxazole-trimethoprim (Septrin); S=streptomycin; CN=gentamicin

Table 4: Antimicrobial resistance patterns of *Enterobacteriaceae* isolates from laying chicken in Ibadan, Nigeria

<b>Antibiotics</b>	<b>Resistance pattern</b>	<b>Number of resistant isolates</b>			
		<i>Escherichia coli</i>	<i>Enterobacter</i> spp	<i>Klebsiella</i> spp	Total isolates
OFX	Mono resistance	2	0	0	2
S	Mono resistance	0	2	0	2
CH	Mono resistance	0	0	1	1
SP	Mono resistance	1	0	0	1
CPX	Mono resistance	0	0	1	1
AU	Mono resistance	0	1	1	2
AM	Mono resistance	1	0	0	1
CN	Mono resistance	0	1	1	2
PEF	Mono resistance	0	0	1	1
SXT	Mono resistance	1	0	0	1
OFX-S	Double resistance	0	2	0	2
OFX-CH	Double resistance	0	0	2	2
OFX-CPX	Double resistance	1	0	0	1
OFX-AU	Double resistance	0	1	0	1
OFX-AM	Double resistance	1	0	0	1
OFX-CN	Double resistance	0	1	0	1
OFX-SXT	Double resistance	1	0	0	1
OFX-S-CH	MDR (Triple resistance)	1	1	0	2
OFX-S-AU	MDR (Triple resistance)	0	0	1	1
OFX-S-AM	MDR (Triple resistance)	0	1	0	1
OFX-S-SXT	MDR (Triple resistance)	1	0	0	1
OFX-S, CH-AU	MDR (Quadruple resistance)	1	0	0	1
OFX-S, CH-AM	MDR (Quadruple resistance)	0	0	1	1
OFX-S-CH-SXT	MDR (Quadruple resistance)	0	1	0	1
S-CH-CPX-AU	MDR (Quadruple resistance)	0	1	1	2
S-CH-CPX-AM	MDR (Quadruple resistance)	1	0	0	1
S-CH-CPX-AU-SXT	MDR (Quintuple resistance)	0	1	0	1
OFX-S-CH-AU-SXT	MDR (Quintuple resistance)	2	0	0	2
S-CH-AM-PEF-SXT	MDR (Quintuple resistance)	7	4	2	13
<b>Total</b>		<b>21</b>	<b>17</b>	<b>12</b>	<b>50</b>

A total of 29 resistance patterns were observed in 50 resistant *Enterobacteriaceae* isolates, with 10 mono-resistance (14 isolates, 28.0%), 7 double resistance (9 isolates, 18.0%) and 12 multi-drug resistance patterns (27 isolates, 54.0%). AM=amoxicillin; AU=amoxicillin-clavulanic acid (Augmentin); OFX=ofloxacin (Tarivid); SP=Sparfloxacin; CPX=ciprofloxacin; PEF=pefloxacin; CH=chloramphenicol; SXT=sulfamethoxazole-trimethoprim (Septrin); S=streptomycin; CN=gentamicin; MDR= Multidrug resistance

#### Antibiotic susceptibility test results:

*Escherichia coli* isolates showed 78.2% sensitivity to ciprofloxacin, 73.4% to ofloxacin, 71.8% to sparfloxacin, and 70.9% to pefloxacin but resistant to sulfamethoxazole-trimethoprim (73.4%), streptomycin (65. 4%), and to other antibiotics (63.7%). About 22% of *Klebsiella pneumoniae* were susceptible to gentamicin, 33.3% to ofloxacin, 33.3% to ciprofloxacin, and 44.5% to other antibiotics. *Enterobacter* spp were resistant to ofloxacin (100%), sulfamethoxazole-trimethoprim (84.5%), chloramphenicol (68.8%), gentamicin (64.1%), ciprofloxacin (60.9%) while being sensitive to amoxicillin-clavulanic acid (93.8%), pefloxacin (70.3%) and streptomycin (70.3%) (Table 3).

A total of 29 resistance patterns were observed in 50 resistant *Enterobacteriaceae* isolates, with 10 mono-resistance (14 isolates,

28.0%), 7 double resistance (9 isolates, 18.0%) and 12 MDR patterns (27 isolates, 54.0%), with MDR patterns varying from resistance to 3 to 5 antibiotic classes (Table 4).

#### Discussion:

Antimicrobial drugs are commonly used in poultry, livestock, and aquaculture at non-therapeutic dosages as growth promoters and prophylaxis in feeds and water for protracted periods thereby contributing to the development and spread of resistant organisms (8). Additionally, the misuse of antimicrobials and other drugs in household chickens may promote the zoonotic transmission of AMR pathogens, thereby posing a threat to animal and human health (10). The failure to complete the prescribed course of antimicrobials or the preservation of medication for future use in

chickens and humans can lead to inappropriate usage, which could trigger the development of resistance in bacterial strains (19). The over-reliance on antibiotics such as tetracycline as growth promoters in chickens can also lead to the development of resistance, and consuming poultry products can transmit antimicrobial resistance to humans. However, in Nigeria, there has been report of circulation of *Enterobacteriaceae* strains among chickens and animal populations (7).

In this study, the overall prevalence of the chicken colonization by *Enterobacteriaceae* was 95.0% (190/200) with isolation of three members of this family; *E. coli* in 60.5% (121/200), *Enterobacter* spp in 27.9% (46/200) and *Klebsiella pneumoniae* in 11.5% (23/200) from commercial laying chickens in the 10 LGAs studied. Regrettably, poultry farms have contributed to environmental contamination with antibiotic-resistant bacteria, primarily members of the family *Enterobacteriaceae*, which are transmitted to chickens, livestock, and humans through direct contact or through contaminated food products (20,21). Similar studies carried out in Nigeria in 2011 and 2019 showed comparatively similar results (7,22). Furthermore, Ibrahim et al., (23) reported *E. coli* as a common enteric pathogen, specific strains of which can cause human and animal disease.

In the current study, *E. coli* was the most frequently recovered bacteria in 59.6% (171/287). This might be due to poor hygiene practices and sanitation status, as well as lack of biosecurity in the farms, consistent with previous research conducted in Ethiopia (24). Most of the previous studies demonstrated that the frequency of *E. coli* from chicken faeces could vary greatly with the time of sample collection, chicken age, and the diet (21). The high prevalence of bacteria from the *Enterobacteriaceae* family is a significant problem for commercial poultry breeders, as reported by Khouja et al (13).

The *Enterobacteriaceae* isolates in our study were sensitive to ciprofloxacin, ofloxacin, sparfloxacin, and pefloxacin but were largely resistant to cotrimoxazole, streptomycin, and other antibiotics. According to Awogbemi et al., (14), *E. coli* isolated from Portuguese poultry were reported to be highly resistant to tetracycline (70.0%) and ampicillin (63.0%) while low level of resistance was observed with co-trimoxazole (33.0%), gentamicin (17.0%) and co-amoxiclav (17%). Our study agreed with the previous work done by Kaushik et al., (25), where the growing threat of MDR observed more frequently in some Gram-negative bacteria such as *E. coli* continues to cause concern.

Antimicrobial resistant bacteria are detected in poultry wastes, poultry products, and poultry environments. Humans through

consumption of contaminated poultry products (7,14), may acquire these resistant bacterial strains. Our study isolated and identified a high number of AMR pathogenic bacteria from cloacae swabs, showing that these bacteria could contaminate poultry meats and other poultry products.

## Conclusion:

This present study showed high rates of faecal colonization of commercial poultry chickens in Ibadan, southwest Nigeria with MDR *Enterobacteriaceae* isolates (*E. coli*, *Enterobacter* spp., and *K. pneumoniae*). The high prevalence of these MDR isolates may be attributed to poor hygiene, improper use of biosecurity measures, poor sanitation, and excessive administration of antibiotics in poultry production. Our study recommends the need to ensure appropriate use of antibiotics in poultry production in order to prevent severe economic implications for poultry production and threats of AMR emergence and spread to the human population.

## Acknowledgements:

The authors appreciated Pan African University Life and Earth Sciences Institute (Including Health and Agriculture), University of Ibadan, Nigeria, and the technical support from the staffs at Laboratory of Microbiology and Parasitology, University of Ibadan.

## Contributions of authors:

OCV was involved in data collection, isolation and write up, while EAA and EBO were involved in research supervision, guidance and correction of the manuscript. All authors approved the manuscript submitted for publication.

## Source of funding:

The research was fully funded by the African Union Commission (AUC) through Pan African University Life and Earth Sciences Institute (Including Health and Agriculture), University of Ibadan, Nigeria.

## Conflict of interest:

No conflict of interest is declared.

## References:

1. Amer, M., Dahshan, A. H., Hassan, H., and Mohamed, A. Studies on the Prevalence of *Enterobacteriaceae* in Chickens and Chicken eggs. *J Vet Med Res.* 2013; 22: 136–144. [doi:10.21608/JVMR.2013.77696](https://doi.org/10.21608/JVMR.2013.77696)
2. Denton, M. *Enterobacteriaceae*. *Int J Antimicrob Agents.* 2017; 29 (Suppl 3): S9–S22. [doi: 10.1016/S0924-8579\(07\)72174-X](https://doi.org/10.1016/S0924-8579(07)72174-X).

3. Agyare, C., Etsiapa Boamah, V., Ngofi Zumbi, C., and Boateng Osei, F. Antibiotic Use in Poultry Production and Its Effects on Bacterial Resistance. In: Kumar, Y (editor). Antimicrobial Resistance - A Global Threat [Internet]. IntechOpen; 2019 [doi:10.5772/intechopen.79371](https://doi.org/10.5772/intechopen.79371)
4. Cadena, M., Kelman, T., Marco, M. L., and Pitesky, M. Understanding antimicrobial resistance (AMR) profiles of *Salmonella* biofilm and planktonic bacteria challenged with disinfectants commonly used during poultry processing. Foods. 2019; 8 (7): 275. [doi:10.3390/foods8070275](https://doi.org/10.3390/foods8070275)
5. Octavia, S., Sara, J., and Lan, R. Characterization of a large novel phage-like plasmid in *Salmonella enterica* serovar Typhimurium. FEMS Microbiol Lett. 2015;362 (8): fnv044 [doi:10.1093/femsle/fnv044/2467663](https://doi.org/10.1093/femsle/fnv044/2467663)
6. Gutierrez, A., De, J., and Schneider, K. R. Prevalence, Concentration, and Antimicrobial Resistance Profiles of *Salmonella* Isolated from Florida Poultry Litter. Journal of Food Protection. 2020; 83 (12): 2179-2186. [doi:10.4315/JFP-20-215](https://doi.org/10.4315/JFP-20-215)
7. Johnson, S., Bugyei, K., Nortey, P., and Tasiame W. Antimicrobial drug usage and poultry production: case study in Ghana. Anim Prod Sci. 2019; 59 (1): 177-182. [doi:10.1071/AN16832](https://doi.org/10.1071/AN16832)
8. Marshall, B. M., and Levy, S. B. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev. 2011; 24 (4): 718-733. [doi:10.1128/CMR.00002-11](https://doi.org/10.1128/CMR.00002-11)
9. Oliveira, J. M., Cardoso, M. F., Moreira, F. A., and Müller, A. Phenotypic antimicrobial resistance (AMR) of avian pathogenic *Escherichia coli* (APEC) from broiler breeder flocks between 2009 and 2018. Avian Pathol. 2022; 51 (4): 388-394. [doi:10.1080/03079457.2022.2074816](https://doi.org/10.1080/03079457.2022.2074816)
10. Wang, X. W., Cao, Y. M., and Park, C. The relationships among community experience, community commitment, brand attitude, and purchase intention in social media. Int J Informat Manag. 2019; 49: 475-488. [doi:10.1016/j.ijinfomgt.2019.07.018](https://doi.org/10.1016/j.ijinfomgt.2019.07.018)
11. Zhu, M., Wang, L., Zhuge, Z., et al. Risk Factors Associated with Multi-Drug Resistance in Neonatal Sepsis Caused by *Escherichia coli*. Infect Drug Resist. 2023; 16: 2097-2106. [doi:10.2147/IDR.S403135](https://doi.org/10.2147/IDR.S403135)
12. Exner, M., Bhattacharya, S., Christiansen, B., et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? GMS Hyg Infect Control. 2017; 12: Doc05 [doi:10.3205/dgkh000290](https://doi.org/10.3205/dgkh000290)
13. Khouja, T., Mitsantisuk, K., Tadrous, M., and Suda, K. J. Global consumption of antimicrobials: impact of the WHO Global Action Plan on Antimicrobial Resistance and 2019 coronavirus pandemic (COVID-19). J Antimicrob Chemother. 2022; 77 (5): 1491-1499. [doi:10.1093/jac/dkac028](https://doi.org/10.1093/jac/dkac028).
14. Awogbemi, J., Adeyeye, M., and Akinkunmi, E. O. A Survey of Antimicrobial Agents Usage in Poultry Farms and Antibiotic Resistance in *Escherichia coli* and staphylococci Isolates from the Poultry in Ile-Ife, Nigeria. J Infect Dis Epidemiol 2018; 4 (1). [doi:10.23937/2474-3658/1510047](https://doi.org/10.23937/2474-3658/1510047)
15. Adeboye, O. The City of Ibadan: Yoruba Towns and Cities. Bookshelf Resources Ltd, Ibadan; 2003. [https://www.researchgate.net/publication/344044327\\_The\\_City\\_of\\_Ibadan](https://www.researchgate.net/publication/344044327_The_City_of_Ibadan)
16. Tijani, M. K., Köster, P. C., Guadano-Procesi, I., et al. High Diversity of *Giardia duodenalis* Assemblages and Sub-Assemblages in Asymptomatic School Children in Ibadan, Nigeria. Trop Med Infect Dis. 2023; 8(3): 152. [doi:10.3390/tropicalmed8030152](https://doi.org/10.3390/tropicalmed8030152)
17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. CLSI, 2008.
18. Magiorakos, A. P., Srinivasan, A., Carey, R. B., et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18 (3): 268-281. [doi:10.1111/j.1469-0691.2011.03570.x](https://doi.org/10.1111/j.1469-0691.2011.03570.x)
19. Barton, M. D. Antibiotic use in animal feed and its impact on human health. Nutr Res Rev. 2000; 13 (2):279-299. [doi:10.1079/095442200108729106](https://doi.org/10.1079/095442200108729106).
20. Moawad, A. A., Hotzel, H., Neubauer, H., et al. Antimicrobial resistance in *Enterobacteriaceae* from healthy broilers in Egypt: emergence of colistin-resistant and extended-spectrum β-lactamase-producing *Escherichia coli*. Gut Pathog. 2018; 10 (1): 39. [doi:10.1186/s13099-018-0266-5](https://doi.org/10.1186/s13099-018-0266-5)
21. Nhung, N. T., Chansiripornchai, N., and Carrique-Mas, J. J. Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review. Front Vet Sci. 2017; 4. [doi:10.3389/fvets.2017.00126](https://doi.org/10.3389/fvets.2017.00126)
22. Olatoye, O. I. Antibiotics use and resistance patterns of *Salmonella* species in poultry from Ibadan, Nigeria. Trop Veterinar. 2011; 29 (1): 28-35.
23. Ibrahim, R. A., Cryer, T. L., Lafi, S. Q., Basha, E. A., Good, L., and Tarazi, Y. H. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Vet Res. 2019; 15 (1): 1-16. [doi:10.1186/s12917-019-1901-1](https://doi.org/10.1186/s12917-019-1901-1)
24. Abebaw, A., Tesera, H., Belachew, T., and Mihiret, G. D. The bacterial profile and antibiotic susceptibility pattern among patients with suspected bloodstream infections, Gondar, north-west Ethiopia. Pathol Lab Med Int. 2018; 10: 1-7. [doi:10.2147/PLMI.S153444](https://doi.org/10.2147/PLMI.S153444)
25. Kaushik, M., Kumar, S., Kapoor, R. K., Virdi, J. S., and Gulati, P. Integrons in *Enterobacteriaceae*: diversity, distribution, and epidemiology. Int J Antimicrob Agents. 2018; 51 (2): 167-176. [doi:10.1016/j.ijantimicag.2017.10.004](https://doi.org/10.1016/j.ijantimicag.2017.10.004)