ANTIMICROBIAL RESISTANCE OF ENTEROBACTERIACEAE ISOLATED FROM SHELL EGGS IN OWERRI WEST, IMO STATE, NIGERIA

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Abstract

Poultry eggs represent one of the most widely consumed sources of animal protein across the globe, yet eggshell surfaces can serve as reservoirs for pathogenic bacteria that pose serious public health risks when contaminated with antibiotic resistant organisms. This study examined the presence and antimicrobial susceptibility patterns of Enterobacteriaceae isolated from shell eggs obtained from two commercial chicken farms in Owerri West Local Government Area, Imo State, Nigeria. A total of 100 fresh eggs (50 per farm) were subjected to standard bacteriological analysis. Bacterial isolates were identified through colony morphology, Gram staining, and biochemical characterization using IMViC, catalase, oxidase, urease, and motility tests. Antimicrobial susceptibility was assessed using the Kirby Bauer disk diffusion method against ten commonly used antibiotics. Two Gram negative Enterobacteriaceae were provisionally identified as *Enterobacter* spp. and *Klebsiella pneumoniae*. Total bacterial counts varied between sampling locations, with eggs from Location A recording substantially higher counts (7.0×10^4 CFU/mL) than those from Location B (1.0×10^4 CFU/mL). Ciprofloxacin demonstrated the strongest inhibitory activity among all tested antibiotics, while resistance was observed towards augmentin, gentamicin, and nalidixic acid in *K. pneumoniae*. The findings underscore the potential role of eggshells as reservoirs of antimicrobial resistant bacteria and highlight the necessity for improved hygiene practices, regular surveillance, and strengthened biosecurity measures in poultry production systems within the One Health framework.

Keywords: *Enterobacteriaceae; Eggshell contamination; Antimicrobial resistance; Poultry eggs; Ciprofloxacin; One Health; Food safety*

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most pressing threats to global public health in the twenty first century. The rapid proliferation of resistant microbial strains has substantially diminished the therapeutic effectiveness of antibiotics used in both human and veterinary medicine, complicating the clinical management of infectious diseases and contributing to increased morbidity, mortality, and healthcare expenditure (Laxminarayan *et al.*, 2016; World Health Organization, 2021). The World Health Organization has identified the misuse and overuse of antimicrobial agents in food producing animals as a critical driver of the emergence and dissemination of resistant bacterial populations (World Health Organization, 2021).

Poultry production constitutes a significant component of global food systems, providing an accessible and reasonably priced supply of high quality animal protein in the form of meat and eggs. Eggs, in particular, are consumed in large quantities owing to their high nutritional content and essential nutrient profile required for human growth and development (Alba *et al.*, 2015). In many developing countries, including Nigeria, eggs are frequently sold through informal retail channels where hygienic handling standards may be inadequate and regulatory compliance remains lax (Adabara *et al.*, 2020).

Members of the family Enterobacteriaceae rank among the most commonly isolated bacterial groups associated with poultry and poultry products. This family of Gram negative bacteria encompasses several genera of medical and veterinary importance, including *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter*, and *Proteus*, many of which are capable of causing foodborne infections in humans (Janda and Abbott, 2014). Poor sanitation during egg production, collection, transportation, and marketing can significantly increase the risk of microbial contamination of eggshell surfaces (Adabara *et al.*, 2020). Recent evidence has shown that eggshell contamination is frequently attributable to direct contact with fecal materials, litter, nesting surfaces, dust, and other contaminants prevalent in poultry housing environments (Hinson *et al.*, 2025).

The presence of antimicrobial resistant bacteria on eggshells is therefore a significant public health concern, particularly in regions where eggs are handled without adequate sanitation protocols. These shell associated bacteria are not merely indicators of fecal contamination but may also function as vectors for the horizontal transfer of antimicrobial resistance genes, thereby contributing to the wider dissemination of resistance determinants through the food chain (Kamel *et al.*, 2024; Hinson *et al.*, 2025). The objective of this study was to investigate the occurrence of Enterobacteriaceae on shell eggs obtained from poultry farms in Owerri West Local Government Area of Imo State, Nigeria, and to evaluate the antimicrobial susceptibility patterns of the isolated bacterial strains.

2. Materials and Methods

2.1 Study Area

The study was conducted in Owerri West Local Government Area, one of the 27 Local Government Areas in Imo State, Nigeria. Imo State is situated in the South Eastern part of Nigeria and covers an area of approximately 5,530 square kilometres. The state shares borders with Enugu and Ebonyi States to the north, Anambra State to the west, Rivers State to the south, and Cross River and Akwa Ibom States to the east. Imo State derives its name from the Imo River, which originates from the Okigwe and Awka uplands. The study area lies within latitude 4°45′N to 7°15′N and longitude 6°50′E to 7°25′E.

2.2 Sample Collection

A total of 100 fresh table eggs were collected from two commercial poultry farms located in Owerri West Local Government Area. The farms were selected on the basis of their large scale production capacity and accessibility. Fifty eggs were randomly collected from each farm, yielding a total sample size of 100 eggs. Only visibly clean, intact, and undamaged eggs without obvious fecal contamination were selected for inclusion in the study. The eggs were carefully placed in sterile plastic containers, transported to the laboratory under aseptic conditions, and analysed within 24 hours of collection to prevent changes in microbial load. Sterile gloves were used throughout the handling process to minimize external contamination.

2.3 Bacteriological Analysis

Eggshell samples were processed using standard microbiological procedures for the isolation and enumeration of bacteria. Each egg was aseptically rinsed to recover microorganisms present on the shell surface. Individual eggs were placed in sterile sample bags containing 90 mL of sterile buffered peptone water and gently shaken to dislodge microorganisms. A tenfold serial dilution of the rinse solution was prepared using sterile distilled water. From appropriate dilutions (10^{-3} and 10^{-5}), 0.1 mL aliquots were aseptically inoculated onto sterile agar plates using the spread plate technique. The inoculated plates were incubated for 24 hours at 37°C. Total Viable Count (TVC) and Total Coliform Count (TCC) were determined and expressed as colony forming units per millilitre (CFU/mL). MacConkey agar was used for the selective isolation of coliform bacteria, while nutrient agar was employed for the enumeration of total heterotrophic bacteria. All analyses were carried out in triplicate, and results were expressed as mean values ± standard deviation.

2.4 Bacterial Identification

Distinct colonies obtained from the culture plates were subcultured to obtain pure isolates. The isolates were then identified on the basis of colony morphology, Gram staining characteristics, and a battery of biochemical tests. Gram staining was performed following standard protocols: smears were stained with crystal violet for one minute, treated with Gram's iodine as a mordant, decolourized using 95% ethanol, and counterstained with safranin for 30 seconds. The slides were examined under a light microscope using oil immersion (×100 objective) to determine Gram reaction and cell morphology.

Biochemical tests included the Indole test for tryptophan degradation, the Methyl Red test for stable acid production during glucose fermentation, the Voges Proskauer test for acetoin production, and the Citrate utilization test on Simmons citrate agar. Additional tests included urease activity on urea agar slants, catalase activity using 3% hydrogen peroxide, oxidase reaction on filter paper, motility assessment, and amylase production on starch agar plates flooded with iodine solution.

2.5 Antibiotic Susceptibility Testing

The antimicrobial susceptibility of the bacterial isolates was determined using the Kirby Bauer disk diffusion method on Mueller Hinton agar following standard microbiological guidelines. A standardized bacterial suspension equivalent to 0.5 McFarland turbidity standard was prepared from pure cultures and uniformly spread over the surface of Mueller Hinton agar plates using sterile swabs. Commercial antibiotic discs were placed on the inoculated agar surface using sterile forceps. The plates were incubated at 37°C for 18 to 24 hours. After incubation, the diameters of zones of inhibition around each antibiotic disc were measured in millimetres using a ruler. The susceptibility of the isolates to each antibiotic was interpreted as susceptible, intermediate, or resistant according to standard interpretive guidelines. Data obtained from the study were analysed using descriptive statistics and expressed as mean ± standard deviation. Differences between bacterial counts from the two sampling locations were evaluated using the Student's t test at a significance level of $p < 0.05$.

3. Results

3.1 Biochemical Characteristics of Bacterial Isolates

The biochemical profiles of the bacterial isolates recovered from shell eggs collected from the two poultry farms are presented in Table 1. Both isolates displayed creamy colonies on nutrient agar and exhibited rod shaped morphology under microscopic examination. Gram staining confirmed that both isolates were Gram negative rods, consistent with the characteristics of members of the Enterobacteriaceae family. Both isolates demonstrated positive responses for the fermentation of glucose, lactose, and sucrose with gas production, indicating their capacity to metabolize carbohydrates. Neither isolate produced hydrogen sulphide, and both exhibited positive catalase activity and citrate consumption.

The two isolates differed in several key biochemical properties. Isolate A tested negative for urease activity and motility, while Isolate B demonstrated positive urease activity and was motile. The Voges Proskauer reaction was negative for Isolate A but positive for Isolate B. Based on the overall biochemical profile, Isolate A was provisionally classified as *Enterobacter* spp., whereas Isolate B was identified as *Klebsiella pneumoniae*. These findings indicate the presence of members of the Enterobacteriaceae family on eggshells from the sampled farms, organisms that are frequently linked to fecal contamination and environmental exposure in poultry production systems (Janda and Abbott, 2014).

Table 1. Biochemical characteristics of bacterial isolates obtained from shell eggs.

Test	Isolate A	Isolate B
Colony morphology	Creamy colonies	Creamy colonies
Cell shape	Rod	Rod
Gram reaction	Negative	Negative
Glucose fermentation	+	+
Lactose fermentation	+	+
Sucrose fermentation	+	+
Gas production	+	+
H■S production	–	–
Citrate utilization	+	+
Catalase test	+	+
Indole test	–	–
Amylase test	+	+
Urease test	–	+
Motility	–	+
Oxidase test	–	–
Voges Proskauer	–	+
Methyl Red	+	–
Probable organism	Enterobacter spp.	K. pneumoniae

3.2 Morphological Characteristics

Table 2 summarises the morphological characteristics of the bacteria isolated from eggshell samples collected from the two locations. Colonies cultured on MacConkey agar displayed a

pinkish hue, indicating lactose fermentation, a characteristic trait of coliform bacteria. Isolate A produced irregularly shaped colonies ranging from 3 to 8 mm in diameter with raised or flat elevations, while Isolate B formed regularly shaped colonies with diameters of 2 to 4 mm and raised elevations. These morphological differences suggest that the samples from the two farms harboured distinct bacterial species or strains.

Table 2. Morphological characteristics of bacteria isolated from eggshell samples.

Parameter	Location A	Location B
Culture medium	MacConkey agar	MacConkey agar
Colony colour	Pinkish	Pinkish
Colony size (mm)	3–8 mm	2–4 mm
Colony shape	Irregular	Regular
Colony elevation	Raised/Flat	Raised

The presence of lactose fermenting colonies on MacConkey agar further confirms the likely presence of coliform bacteria, which are widely used as indicators of fecal contamination and environmental hygiene status on eggshell surfaces. Such contamination is frequently attributed to contact with fecal materials, litter, nesting surfaces, dust, and other contaminants in poultry housing environments, as reported in recent studies of microbial quality in poultry eggshells and egg facilities (El Ftouhy *et al.*, 2022; Hinson *et al.*, 2025).

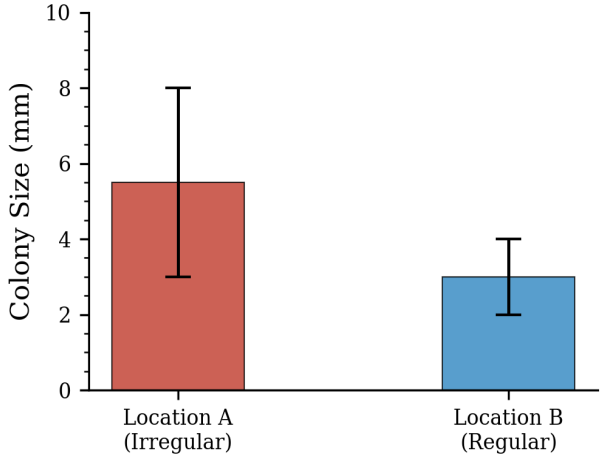

Figure 1: *Colony size distribution of bacterial isolates from Location A and Location B.*

3.3 Total Bacterial Counts

The total bacterial counts obtained from eggshells collected from the two locations are presented in Table 3 and illustrated in Figure 2. Eggs obtained from Location A exhibited a substantially higher bacterial load (7.0×10^4 CFU/mL) compared with those from Location B (1.0×10^4 CFU/mL), with a standard error of the mean (SEM) of 1.69×10^4. This difference highlights the variability in microbial contamination of eggshells across farms, which may reflect differences in management practices and environmental hygiene within these production systems.

Table 3. Total bacterial counts of isolates recovered from eggshell samples.

Location	Total Bacterial Count (CFU/mL)
A	7.0 × 10■
B	1.0 × 10■
SEM	1.69 × 10■

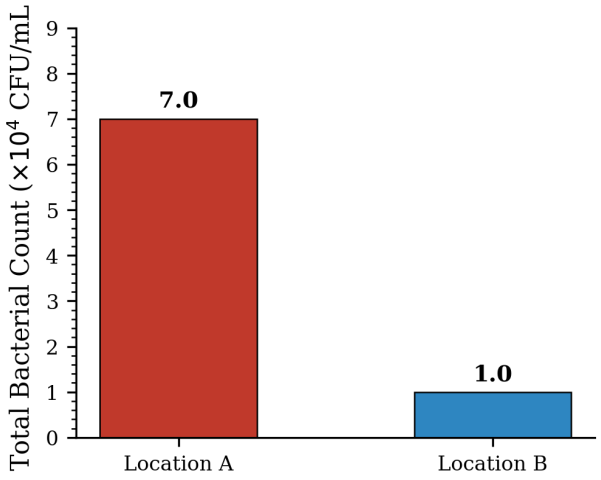

Figure 2: *Total bacterial counts from eggshell samples at Location A and Location B.*

3.4 Antibiotic Susceptibility Profile

The antimicrobial susceptibility patterns of the bacterial isolates against ten selected commercial antibiotics are presented in Table 4 and graphically illustrated in Figures 3 and 4. The results revealed varying degrees of susceptibility among the tested antibiotics against *Enterobacter* spp. and *Klebsiella pneumoniae*.

Table 4. Antibiotic susceptibility profile of bacterial isolates (zone of inhibition in mm).

Antibiotic	Enterobacter spp.	K. pneumoniae	SEM
Tarivid (Ofloxacin)	18	19	0.50
Reflacine (Pefloxacin)	11	22	5.50
Ciprofloxacin	21	27	3.00
Augmentin	14	–	–
Gentamycin	20	–	–
Streptomycin	18	16	1.00
Ceporex (Cephalexin)	20	19	0.50

Nalidixic acid	16	–	–
Septrin (Cotrimoxazole)	16	14	1.00
Ampicillin	20	22	1.00

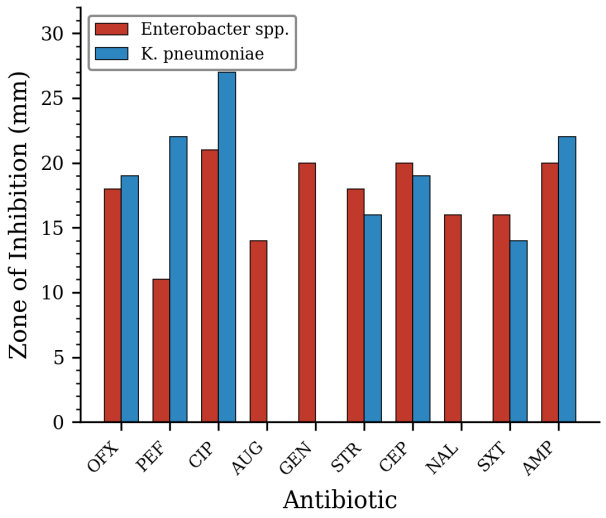

Figure 3: *Zones of inhibition (mm) of selected antibiotics against Enterobacter spp. and K. pneumoniae isolated from shell eggs.*

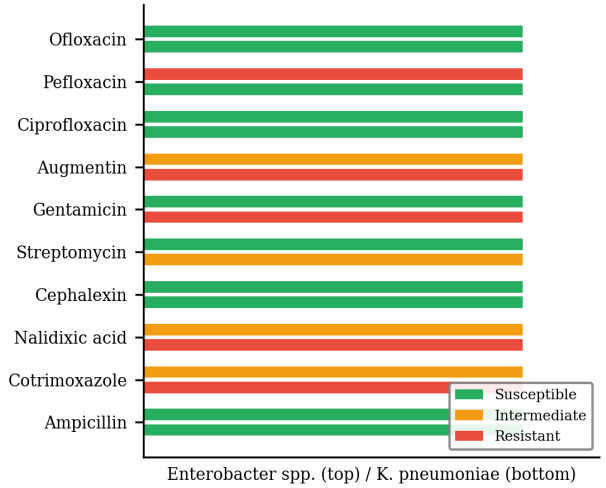

Figure 4: *Antimicrobial resistance profile of isolates showing susceptible (green), intermediate (yellow), and resistant (red) responses.*

4. Discussion

The present study provides compelling evidence that raw eggshells from commercial poultry farms in Owerri West, Imo State, harbour bacterial contaminants belonging to the Enterobacteriaceae family, specifically *Enterobacter* spp. and *Klebsiella pneumoniae*. The isolation of these organisms from eggshell surfaces is consistent with earlier reports that eggshells can act as vehicles for the transmission of potentially pathogenic bacteria from the farm environment to the consumer (Adabara *et al.*, 2020; El Ftouhy *et al.*, 2022). These findings carry significant implications within the One Health framework, which recognizes the interconnection between animal health, environmental conditions, and human wellbeing.

The marked difference in total bacterial counts between the two sampling locations, with Location A recording a sevenfold higher load than Location B, likely reflects disparities in farm management, environmental hygiene, and biosecurity implementation. Higher bacterial loads on eggshell surfaces have been strongly associated with poor sanitation, accumulation of fecal materials, and inadequate biosecurity measures, particularly in settings where hygiene practices during egg laying, collection, and handling are not rigorously applied (Hemeda *et al.*, 2025). The elevated counts observed at Location A suggest that this particular farm may benefit from targeted interventions aimed at improving nest box hygiene, litter management, and egg collection protocols. Similar observations have been reported in studies examining microbial contamination patterns across poultry farms with differing levels of sanitary compliance in tropical settings (Hinson *et al.*, 2025).

The identification of *Klebsiella pneumoniae* on eggshell surfaces is of particular concern from a public health standpoint, given the well documented propensity of this species to acquire and disseminate antimicrobial resistance determinants (Kamel *et al.*, 2024). *Klebsiella pneumoniae* is recognized as a member of the ESKAPE group of pathogens, which are responsible for a substantial proportion of healthcare associated infections worldwide. The presence of this organism on eggshells indicates a potential route of transmission from poultry environments to the human food chain, with implications for the amplification of resistance genes across ecological niches.

The antibiotic susceptibility data revealed that ciprofloxacin produced the largest zones of inhibition, measuring 21 mm against *Enterobacter* spp. and 27 mm against *K. pneumoniae*, indicating strong antibacterial activity. This observation aligns with recent reports showing that fluoroquinolones, particularly ciprofloxacin, retain significant effectiveness against Gram negative poultry isolates compared with older antibiotic classes (Kamel *et al.*, 2024; Hinson *et al.*, 2025). The continued efficacy of ciprofloxacin in this study is encouraging, though it should be interpreted with caution given the global trend of increasing fluoroquinolone resistance among Enterobacteriaceae from livestock sources.

Similarly, gentamicin and cephalexin demonstrated relatively high inhibitory effects against *Enterobacter* spp., reflecting their continued utility against some Enterobacteriaceae despite global increases in resistance. In contrast, pefloxacin produced smaller zones of inhibition, especially against *Enterobacter* spp. (11 mm), indicating

reduced effectiveness against this isolate. The absence of measurable zones for augmentin, gentamicin, and nalidixic acid against *K. pneumoniae* indicates resistance to these agents, which is a finding that warrants further investigation through molecular characterization of resistance mechanisms.

Moderate activity was observed for cotrimoxazole, streptomycin, and ampicillin, which may reflect both intrinsic and acquired resistance traits documented among Enterobacteriaceae from poultry sources (Kamel *et al*., 2024). The variable susceptibility patterns observed across the tested antibiotics are consistent with findings from systematic reviews examining antimicrobial resistance profiles of Enterobacteriaceae from eggs and poultry products globally (Hinson *et al*., 2025). The selective pressure from routine antimicrobial use in poultry farming can drive the emergence and spread of resistant strains, and the results of this study underscore the importance of rational antibiotic stewardship in livestock production.

The observed variability in antibiotic susceptibility also emphasizes the role of environmental and management factors in shaping resistance phenotypes at the farm level. Farms that employ prophylactic or growth promotional antibiotic use are more likely to harbour resistant bacterial populations on eggshell surfaces, as selective pressure favours the survival and proliferation of resistant clones (Laxminarayan *et al*., 2016). The implications of these findings extend beyond the immediate farm setting, as resistant bacteria shed onto eggshells may enter the human food chain through handling, consumption of undercooked eggs, or cross contamination in domestic kitchens.

From the perspective of food safety policy, the findings of this study reinforce the need for an integrated surveillance system that monitors antimicrobial resistance at multiple points along the poultry value chain, from production through retail to consumption. Such an approach is in line with the One Health paradigm, which advocates for coordinated action across the animal, human, and environmental health sectors to address the challenge of AMR (World Health Organization, 2021). The implementation of good hygiene practices (GHP), hazard analysis critical control point (HACCP) protocols, and regular microbial monitoring programmes in poultry farms could substantially reduce the risk of eggshell contamination and limit the dissemination of resistant organisms to consumers.

5. Conclusion

This study demonstrates that eggshells from commercial poultry farms in Owerri West, Imo State, Nigeria, are contaminated with potentially pathogenic Enterobacteriaceae, including *Enterobacter* spp. and *Klebsiella pneumoniae*. Significant differences in bacterial load were observed between the two sampled farms, reflecting disparities in hygiene and biosecurity practices. The antimicrobial susceptibility patterns revealed that while some antibiotics, particularly ciprofloxacin, remain effective, resistance to commonly used antibiotics is emerging. Notably, *K. pneumoniae* showed resistance to augmentin, gentamicin, and nalidixic acid, highlighting the potential for resistant bacteria to enter the human food chain through contaminated eggshells.

These findings highlight the necessity for enhanced farm biosecurity, strict hygiene practices during egg production and handling, rational use of antibiotics in poultry production systems, and ongoing surveillance of bacterial contamination and resistance patterns. Within the One Health concept, the implementation of these measures is critical to reducing the risk of foodborne illnesses and curbing the dissemination of antimicrobial resistant bacteria to consumers. Future research should employ molecular methods for definitive species identification and resistance gene characterization, and should expand the geographic scope to provide a more representative picture of eggshell contamination patterns across Nigeria.

Conflict of Interest

The authors declare no conflict of interest related to the publication of this manuscript. The research was entirely self funded; no external funding was received from any funding agency.

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