105 (2026) 106708

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Full-Length Article

# The effect of papain on some bacterial pathogens in poultry meat

Hanan S. Khalefa [a,*], Zeinab S. Ahmed [b], Amr Abd El-khalik A. El-Saadany [c], Dina Ismail Elzahaby [d], Mona M. Abd El-Fattah [e], Enas A. Shedeed [f], Nesma M. Kamel [g], Eman.F.E. Shebl [h], May F. Abdelaty [i], Amani Abd El Latif Mosleh [j]

[a] Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[b] Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[c] Department of Bacteriology, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Tanta Branch, Egypt
[d] Department of Food Hygiene, Animal Health Research Institute, Agricultural Research Center (ARC), Shebin El Kom branch, Egypt
[e] Department of Food Hygiene, Animal Health Research Institute(AHRI), Agriculture Research Center (ARC), Shebin El-Kom Branch, Egypt
[f] Department of Bacteriology, Animal Health Research Institute, Agricultural Research Center (ARC), Eldokki, Giza, Egypt
[g] Researcher, Reference Laboratory of Veterinary Quality Control on Poultry Production, Animal Health Research Institute (AHRI), Agricultural research center (ARC), Egypt
[h] Department of Poultry Diseases, Animal Health Research Institute, Agricultural Research Center (ARC), Shebin El Kom Branch, Egypt
[i] Senior Researcher, Reference Laboratory for Quality Control on Poultry Production, Animal Health Research Institute, Agricultural Research Center (ARC), Egypt
[j] Department of Bacteriology, Animal Health Research Institute, Agricultural Research Center (ARC), Shebin El Kom branch, Egypt

ARTICLE INFO

*Keywords:*
Antimicrobial resistance
ESBL
*E. coli*
Turkey
One health

ABSTRACT

The poultry sector is a significant reservoir of antimicrobial-resistant (AMR) bacteria, posing a substantial threat to public health and food safety. This study quantified farm-level contamination in chickens and turkeys, characterized *Escherichia coli* and *Salmonella* spp. and their ESBL/carbapenemase encoding genes, and evaluated papain as a meat-preservation agent. A total of 442 samples (cloacal swabs, litter, water, feed, worker hands, meat, and organs) were collected from 12 chicken and 8 turkey farms. The total viable count (TVC), total coliform count (TCC) were enumerated. *E. coli* and Salmonella were isolated and tested by multiplex PCR for the detection of ESBL/carbapenemase encoding genes. Antimicrobial resistance (AMR) phenotypes and $bla^{CTX\text{-}M}$ phylogeny were assessed. The effect of papain on chilled chicken meat (12 days) was assessed. The results indicated that microbial loads were higher in chicken than in turkey farms ($p < 0.05$). Chicken litter showed the highest counts (TVC 7.86 ± 0.25; TCC 6.38 ± 0.09 log10 CFU/g). In chickens, *E. coli* prevalence was 18.4% (50/272), varying by sample type ($p < 0.001$), and in turkeys, prevalence was 27.6% (47/170). The ESBL genes in chicken *E. coli* were $bla^{TEM}$ 100%, $bla^{SHV}$ 90%, $bla^{CTX\text{-}M}$ 22%, and $bla^{OXA\text{-}1}$ 10%; and the carbapenemases were $bla^{KPC}$ 14%, $bla^{NDM}$ 10%, and blaVIM 6%. Turkey *E. coli* carried $bla^{TEM}$ (95.7%), $bla^{SHV}$ (85%), $bla^{CTX\text{-}M}$ (21.3%), $bla^{OXA\text{-}1}$ (4.3%), $bla^{KPC}$ (10.6%), $bla^{NDM}$ (10.6%, $bla^{VIM}$ 6.4%. Salmonella occurred in 4.0% of chicken and 18.3% of turkey samples. *Salmonella* isolates with ESBLs from chickens had $bla^{TEM}$ 100%, $bla^{CTX\text{-}M}$ 9%, while $bla^{OXA\text{-}1}$ 0%; in turkeys; $bla^{TEM}$ 90%, and $bla^{OXA\text{-}1}$ 3%. $bla^{KPC}$ was absent in chickens but detected in 13% of turkey isolates. Phylogenetic analyses elucidate the potential transmission pathways. *E. coli* resisted ampicillin/sulbactam (65–67%); Salmonella resisted ceftriaxone (88–91%) and penicillin (82–86%). Papain significantly lowered TVC/TCC/TEC during storage, delaying spoilage. In conclusion, poultry farms, especially turkey farms, harbor multidrug-resistant *E. coli* and Salmonella with clinically important ESBL/carbapenemase genes. Prudent antibiotic use, strengthened biosecurity, and papain-based interventions can mitigate food-safety risks.

## Introduction

Poultry production is a vital component of Egypt's agricultural sector and a cornerstone of national food security. With increasing demand for animal protein, both chicken and turkey farming have expanded significantly in recent years (Shoukry, 2021). Turkey meat, in particular, is favored for its low fat and high protein content, offering a nutritious alternative to red meat. However, turkey production is more sensitive to

* Corresponding author.
*E-mail address:* hanan_saad@cu.edu.eg (H.S. Khalefa).

https://doi.org/10.1016/j.psj.2026.106708
Received 12 December 2025; Accepted 25 February 2026
Available online 26 February 2026

environmental and nutritional stressors than chicken production, and young birds are particularly vulnerable to infectious diseases, especially bacterial infections during the early growth stages (Aslam et al., 2012; Dec et al., 2018). These challenges are compounded by the global threat of antimicrobial resistance (AMR), which is rapidly undermining the effectiveness of common antibiotics in veterinary and human medicine (Olasz et al., 2023).

In the context of poultry health, *Escherichia coli* (*E. coli)* and *Salmonella* spp. are among the most significant bacterial pathogens. Foodborne pathogenic bacteria transmit antimicrobial resistance genes (ARGs). ARGs from farm animals have been reported as the primary source of human infections (Wu et al., 2022). Preventing the spread of AMR bacteria from farm to table is critical for public health. Avian pathogenic *E. coli* (APEC) is responsible for colibacillosis, a systemic infection that causes pericarditis, salpingitis, omphalitis, and arthritis, contributing to high mortality and severe economic losses in poultry farms (Ibrahim et al., 2023; Guabiraba and Schouler, 2015; Shakal et al., 2024; Shalaby et al., 2022; Ahmed et al., 2025). Similarly, *Salmonella* infections, particularly those caused by *S. gallinarum* and *S. pullorum*, lead to fowl typhoid and pullorum disease, respectively, both of which cause systemic illness and production losses (Shakal et al., 2024; Andino and Hanning, 2015; Tariq et al., 2022). Beyond their veterinary importance, both *E. coli* and *Salmonella* spp. pose a serious zoonotic risk, as they can contaminate poultry meat and be transmitted to humans via the food chain.

The indiscriminate and often unregulated use of antibiotics in poultry farming is a major driver of AMR, particularly in low- and middle-income countries (Dadgostar, 2019). This selective pressure has led to the emergence of multidrug-resistant (MDR) strains, many of which carry mobile genetic elements that facilitate horizontal gene transfer. Studies have shown that poultry meat can become contaminated during processing, and even organically raised birds may harbor resistant bacteria due to environmental exposure (Mak et al., 2022; Alam et al., 2020; Khalefa et al., 2025). Among the most concerning resistance mechanisms is the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases, encoded by genes such as $bla^{TEM-1}$, $bla^{SHV-1}$, $bla^{KPC}$, $bla^{NDM}$, and $bla^{VIM}$ (Pitout, 2010; Mughini-Gras et al., 2019; Moawad et al., 2022; Codjoe and Donkor, 2017; Kadry et al., 2021). These enzymes confer resistance to critical antibiotics, including third-generation cephalosporins and carbapenems, agents often reserved as last-line treatments in human medicine.

Considering the growing concern over the spread of AMR through the food chain, it is urgent to implement integrated surveillance and mitigation strategies under the One Health framework and to ensure that hygienic slaughter management is implemented to prevent cross-contamination (Elkholy et al., 2024; Gyawali and Ibrahim, 2014). One promising approach involves the use of natural antimicrobial agents to reduce bacterial contamination in food products. Papain, a cysteine protease derived from the papaya plant (*Carica papaya*), has demonstrated antimicrobial effects in addition to its role in meat tenderization (Karkal et al., 2022; Llerena-Suster et al., 2011). By breaking down bacterial cell wall proteins and disrupting membrane integrity, papain exerts bactericidal effects against a broad spectrum of pathogens, including *E. coli, Salmonella typhimurium, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Eshamah et al., 2014; Zhang et al., 2025). As a safe, biodegradable, and cost-effective alternative, papain could serve as a valuable tool for enhancing poultry meat safety and reducing the risk of human exposure to resistant bacteria.

This study aimed to investigate the prevalence and antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. isolated from various environmental and biological samples collected from chicken and turkey farms in Egypt. Furthermore, it sought to assess the phylogenetic relatedness of the isolates to evaluate potential zoonotic transmission pathways. Additionally, the study evaluated the antibacterial effect of papain, a natural antimicrobial agent, on poultry meat to reduce bacterial load and inhibit the growth of multidrug-resistant organisms.

## Materials and methods

### *Sample collection*

This study was conducted on 20 poultry farms (12 chicken farms and eight turkey farms) located in El-Menoufia Governorate, Egypt, during 2023-2024. All farms reported different morbidity disorders and mortality. A total of 442 samples were aseptically collected, including cloacal swabs ($n = 62$) from apparently healthy or diseased birds (3-5 birds per farm), and environmental samples ($n = 80$), which included litter ($n = 20$), water ($n = 20$), feed ($n = 20$), and pooled hand swabs from workers ($n = 20$; 3 workers per farm). Composite environmental samples were prepared at three to five different locations within each farm. Additionally, on average, five birds per farm were necropsied at various ages, based on morbidity history, as shown in Fig. 1. Three hundred samples of their meat, liver, and hearts were taken under hygienic conditions for microbiological analysis. All samples were transported in an icebox to the laboratory and pre-enriched in 10 mL of buffered peptone water (BPW, Oxoid, UK). Details about farm characteristics and sampling were provided in Table 1.

### *Microbiological examination*

#### *Bacteriological sample preparation*

One milliliter of water samples, worker hand swabs (moistened with sterile saline), and cloacal swabs were mixed with 9 mL of sterile 0.9% saline to prepare a $10^{-1}$ dilution (American Public Health Association (APHA) 2001). For solid samples (litter and feed), 10 g were homogenized in 90 mL of sterile saline (Ismael et al., 2022). Tissue samples (liver, heart, and meat samples from breast muscle) were homogenized using a stomacher (Lab Blender 400, Seward, Model No. AB6021) with 90 mL of 0.1% peptone water (HIMEDIA) for one minute (ISO, 2013). Serial 10-fold dilutions were prepared using 0.9% sterile saline solution.

#### *Enumeration of total viable count (TVC), total coliforms count (TCC), total Enterobacteriaceae count (TEC), and total Staphylococcus count (TSC)*

From the appropriate dilution, 0.1 mL was pipetted into standard plate count agar medium (Difco Laboratories, USA), violet red bile lactose agar (VRBL) medium (HIMEDIA, India), MacConkey agar, and Baird-Parker agar medium (Merck, Germany) containing egg yolk, and spread using a sterile glass spreader for TVC, TCC, TEC, and TSC, respectively. The plates were then incubated at 37 °C for 18 to 24 h. Only plates containing fewer than 300 colony-forming units (CFU) were counted (Danon-Moshe et al., 1985). *Enterobacteriaceae* were represented by reddish purple/pink colonies (APHA, 28). *Staphylococcus aureus* colonies appeared glossy black with a thin white border and a clear zone surrounding them. Colony-forming units (CFU) per gram/mL were calculated using the formula: CFU = dilution × colony count/volume. These values, representing the mean colony-forming units, were converted into logarithmic form ($\log_{10}$ CFU).The traditional plate counting technique detects only viable and culturable bacteria. Stress-adapted or injured cells, as well as microorganisms in a viable-but-non-culturable (VBNC) state, may not grow under the applied culture conditions. Therefore, the reported microbial counts may underestimate the actual contamination level.

#### *Isolation and identification of E. coli and Salmonella spp*

*E. coli*: Samples were pre-enriched in lactose broth (Oxoid, UK) and incubated overnight at 37 °C. A loopful from the broth was streaked on eosin methylene blue (EMB) agar and incubated at 37 °C for 18 to 24 h. Colonies with a green metallic sheen were subcultured for purity and identified using biochemical tests (Quinn et al., 2011).

*Salmonella* spp.: After 18 h of pre-enrichment in BPW, 1 mL was transferred to Rappaport-Vassiliadis (RV) broth and incubated at 42 °C for 24 h. Cultures were streaked on Xylose Lysine Deoxycholate (XLD) agar and incubated at 37 °C for 24 h. Colonies with black centers were


**Fig. 1.** The postmortem examination of freshly necropsied birds shows the following: A: chicken liver with subcapsular hemorrhage and necrosis, B: Airasculaitis, C: recently necropsied turkey with enteric symptoms, D: Postmortem examination of freshly dead turkey shows inflammation of the intestines.

**Table 1**
Details of the studied farms characteristics and sampling.

| Poultry | Farm | Total No. of birds per flock | Age (days) | Ventilation | Types of bedding | Mortality* | Types of samples | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Meat | Liver&heart | Cloacal swaps | Litter | Workers hand | Feed | Water |
| Broilers ***n*** **= 12** | F1 | 4000 | 23 | Semi-closed | Litter | 2.6% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F2 | 15000 | 4 | Closed | Litter | 0.8% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F3 | 4000 | 20 | Semi-closed | Litter | 3% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F4 | 15000 | 34 | Closed | Litter | 0.91% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F5 | 20000 | 29 | Closed | Litter | 1% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F6 | 15000 | 30 | Closed | Litter | 0.39% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F7 | 1500 | 18 | Semi-closed | Litter | 2% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F8 | 35000 | 8 | Closed | Litter | 1.5% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F9 | 30000 | 12 | Closed | Litter | 9% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F10 | 10000 | 26 | Closed | Litter | 2.9% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F11 | 15000 | 22 | Closed | Litter | 7% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| | F12 | 5000 | 2 | Semi-closed | Litter | 4% | 5 | 10 | 4 | 1 | 1 | 1 | 1 |
| Total | | | | | | | 60 | 120 | 44 | 12 | 12 | 12 | 12 |
| Turkey | F1 | 1000 | 80 | Semi-closed | Litter | 0.3% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| *N* = 8 | F2 | 500 | 50 | Opened | Litter | 0.8% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F3 | 100 | 120 | Opened | Litter | 0.62% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F4 | 1000 | 299 | Semi-closed | Litter | 0.83% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F5 | 400 | 19 | Opened | Litter | 0.42% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F6 | 100 | 133 | Opened | Litter | 0.7% | 5 | 10 | 2 | 1 | 1 | 1 | 1 |
| | F7 | 2000 | 55 | Semi-closed | Litter | 1% | 5 | 10 | 3 | 1 | 1 | 1 | 1 |
| | F8 | 1000 | 199 | Semi-closed | Litter | 1.45% | 5 | 10 | 3 | 1 | 1 | 1 | 1 |
| Total | | | | | | | 40 | 80 | 18 | 8 | 8 | 8 | 8 |
| Total no. of all samples | | | | | | | 100 | 200 | 62 | 20 | 20 | 20 | 20 |

presumptively identified as *Salmonella* and confirmed by Gram staining and biochemical tests (Kagirita et al., 2017).

*Molecular identification of carbapenemase- and ESBL-encoding resistance genes in E. coli and Salmonella isolates*

*Genomic DNA extraction*

Genomic DNA was extracted using the boiling method (Murugkar et al., 2003). Two milliliters of enriched culture were centrifuged at 13000 rpm for 10 min. The pellet was resuspended in 200 μL nuclease-free water, heated at 100 °C for 10 min, cooled overnight at 20 °C, and then centrifuged again. The supernatant was used as a DNA template and stored at −20 °C.

*Molecular confirmation for E.coli and Salmonella isolates*

PCR targeting the *16S rRNA* gene (for *E. coli*) and *invA* gene (for Salmonella) was conducted for molecular confirmation using primers from Wang et al. (2002) and referenced protocols (Khalefa et al., 2021).

*Molecular detection of ESBL and carbapenemase encoding resistance genes*

Confirmed isolates were screened for ESBL genes ($bla^{TEM}$, $bla^{SHV}$, $bla^{CTX-M}$, and $bla^{OXA-1}$) and carbapenemase genes ($bla^{KPC,}$ $bla^{NDM}$, and $bla^{VIM}$) using multiplex PCR, as described by Khalefa et al. (2025) and Youseef et al. (2024). PCR products were analyzed using 1% agarose gel

electrophoresis. Primer sequences are listed in Table 2.

*Phylogenetic analysis and sequencing*

Two ESBL-producing *E. coli* isolates (from chicken and turkey) were purified using the QIAquick gel extraction kit (Qiagen, Switzerland). The *CTX-M* gene was sequenced using forward and reverse primers. Sequences were submitted to GenBank BLASTn was used to compare sequences against the NCBI databases. Multiple sequence alignment and phylogenetic analysis were performed using ClustalW and MEGA11 software, applying the neighbor-joining method with 1,000 bootstrap replicates.

*Antibiotic sensitivity test*

The antimicrobial panel used for susceptibility testing was selected according to two primary criteria: (1) inclusion of agents representing the major antimicrobial classes commonly used in poultry production within the study area, and (2) antibiotics essential for identifying ESBL-associated resistance patterns, particularly β-lactams and their therapeutic alternatives. Based on these considerations, the panel comprised fluoroquinolones (ofloxacin, 5 μg), tetracyclines (tetracycline, 30 μg), β-lactams (penicillin, 10 μg; ampicillin/sulbactam, 20 μg), third-generation cephalosporins (ceftriaxone, 30 μg), and lincosamides (clindamycin, 2 μg). These antimicrobials reflect both current and historically prevalent usage in local poultry farms and facilitate the preliminary characterization of multidrug resistance profiles in ESBL-producing *E. coli* and *Salmonella* isolates. Susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, and results were interpreted in accordance with CLSI guidelines (CLSI Clinical and Laboratory Standards Institute, 2020). Isolates exhibiting resistance to three or more antimicrobial classes were categorized as multidrug-resistant (MDR) (Khalefa et al., 2025).

*Antibacterial testing for plant extract*

Papain extract was supplied by the Botany Department, National Research Center, Giza, Egypt. Dried *Carica papaya* leaves were first ground into a fine powder using a laboratory mill. The powdered plant material was then subjected to ethanolic extraction by soaking in 96% ethanol at 40 °C with continuous stirring for 24 h. Following extraction, the mixture was filtered through Whatman No. 1 filter paper to remove plant residues. The filtrate was concentrated using a rotary evaporator at 68 °C under reduced pressure until most of the solvent was removed. The semi-solid residue obtained after solvent evaporation was further dried to yield a crude extract containing papain and associated phytochemical components naturally present in the leaf matrix. For experimental use, the dried crude extract was reconstituted in distilled and deionized water (DDW) to prepare a **5% (w/v) working solution**, corresponding to 5 g of crude extract per 100 mL of DDW. With regard to enzyme potency, the papain solution used in this study was a **crude ethanolic extract** rather than a purified enzymatic preparation. Therefore, its exact papain enzymatic activity was not directly quantified by the supplier. The 5% concentration refers to the **weight of crude extract dissolved per volume of DDW**, which is the standard preparation procedure routinely applied for phytochemical extracts used in antimicrobial or biological assays. The biological activity reported in this study is thus based on crude-extract concentration rather than purified papain activity units (Morshdy et al., 2025).

Salmonella inoculum: *Salmonella typhimurium* was applied at a standardized dose of ~$10^6$ CFU/mL, with 1 mL distributed per fillet.

The experiment: A total of six portions of fresh chicken breast fillets were obtained from a local retail market. Each portion weighed 300 g and was cut into uniform pieces of approximately 5 × 5 cm to standardize surface area and ensure consistent contact with the bacterial suspension and papain solution. Portions were randomly assigned into two treatment groups (three replicates per group). Group 1 (Positive Control); chicken fillets treated with 5 mL sterile distilled water. Group 2 (Papain Treatment): chicken fillets treated with 5 mL of 5% (w/v) papain crude extract prepared as previously described. The bacterial inoculum was pipetted evenly across the chicken meat, which was then stirred with a sterile glass rod to ensure uniform distribution of the bacteria. Over 12 days, samples were stored in sterile polyethylene bags at refrigeration temperature (4 °C) and monitored for bacterial growth. Microbiological assessments (TVC, TCC, and TEC) were conducted every three days. Experiments were conducted in triplicate.

*The statistical analysis*

SPSS version 18.0 was used to analyze the data, and a $p$-value $<0.05$ was considered statistically significant. Multiple categorical samples were compared using chi-square testing. With the "Heatmap" R package (version 4.2.2, R Foundation for Statistical Computing), isolates from various sources were grouped based on their AMR traits. A heatmap library (version 1.0.12) was used to cluster strains (Kolde, 2019).

## Results

*Microbial load in chicken and turkey farms*

Microbiological assessment of 442 samples collected from 12 chicken farms and eight turkey farms revealed that microbial contamination levels were significantly higher in chicken farms than in turkey farms. Among the various sample types analyzed, litter samples from chicken farms exhibited the highest bacterial loads, with total viable counts (TVC), and total coliform counts (TCC), reaching maximum

**Table 2**
Primer sequences for PCR amplification of carbapenemase and ESBL genes.

| | gene | Primer sequence (5'–3') | Band size | Pcr condition | Reference |
|---|---|---|---|---|---|
| ESBLs encoding genes | $bla_{TEM}$ | F: CGC CGC ATA CAC TAT TCT CAG AAT GA R: ACG CTC ACC GGC TCC AGA TTT AT | 445 | initial denaturation for 5 min at 94 °C followed by 30 cycles of denaturation of 1 minute at 61 °C, extension at 72 °C for 1 minute, and final extension at 72 °C for 5 min | (Khalefa et al., 2025; Samir et al., 2022) |
| | $bla_{SHV}$ | F: CTT TAT CGG CCC TCA CTCAA R: AGG TGC TCA TCA TGG GAA AG | 237 | | |
| | $bla_{CTX-M}$ | F: ATG TGC AGY ACC AGTAAR GTK ATG GC R: TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | 593 | | |
| | $bla_{OXA-1}$ | F: ACA CAA TAC ATA TCA ACT TCG C R: AGT GTG TTT AGA ATG GTG ATC | 813 | | |
| carbapenemase encoding genes | *KPC* | F:ATG TCA CTG TAT CGC CGT CT R:TTT TCA GAG CCT TAC TGC CC | 882 | initial denaturation for 5 min at 94 °C followed by 30 cycles of 94 °C for one minute, 55 °C for one minute, and 72 °C for two min and final extension at 72 °C for 5 min. | (Youseef et al., 2024) |
| | *NDM* | F:GGT TTG GCG ATC TGG TTT TC R:CGG AAT GGC TCA TCA CGA TC | 621 | | |
| | *VIM* | F:AGTGGTGAGTATCCGACAG R: ATGAAAGTGCGTGGAGAC | 261 | | |

values of 7.86 ± 0.25 and 6.38± 0.09 $\log_{10}$ CFU/g, respectively. Conversely, internal tissue samples such as liver consistently demonstrated the lowest bacterial counts across both poultry species. In chicken farm environments, TEC was most abundant in cloacal swabs, followed by hand swabs from farm workers and water samples, indicating potential routes for environmental and cross-contamination as shown in Table 3. In turkey farms, the highest microbial loads were noted in litter and worker hand swabs. Internal organs such as the liver and breast meat retained comparatively lower bacterial contamination levels. Statistical analyses demonstrated that microbial counts, including TVC, TCC, and TEC, differed significantly ($p < 0.05$) across various sample types and between the two poultry species. These findings underscore the critical need for targeted hygiene and biosecurity measures in poultry farms to minimize foodborne risks.

*Prevalence of E. coli in poultry and environmental samples*

In chicken farms, *E. coli* was isolated from 50 samples, representing a prevalence of 18.4%, as shown in Table 4. Among environmental samples, the highest recovery rates were obtained from litter, followed by feed and water sources. A statistically significant variation in *E. coli* detection rates was observed across different sample types within chicken farms ($p < 0.001$), indicating the presence of multiple contamination sources and pathways. In turkey farms, *E. coli* was detected in 47 samples, corresponding to a prevalence of 27.6%. Approximately 50% of the turkey *E. coli* isolates originated from litter and feed samples, with the remaining 25% recovered from water samples. Unlike in chicken farms, no statistically significant differences were detected in *E. coli* prevalence among various sample types in turkey farms ($p > 0.05$), suggesting a more uniform pattern of contamination across different sample categories.

*Detection of ESBL and carbapenemase-encoding genes in E. coli isolates*

Multiplex PCR analysis confirmed the presence of ESBL-encoding genes (*blaTEM, bla*$^{SHV,}$ *bla*$^{CTX-M}$, and *bla*$^{OXA-1}$) in the *E. coli* isolates (Table 4). In this study, ESBL-encoding genes *bla*$^{TEM}$, *bla*$^{SHV}$, *bla*$^{CTX-M}$, and *bla*$^{OXA-1}$ were detected in 100% (50/50), 90% (45/50), 22% (11/50), and 10% (5/50) of the 50 isolates from chicken farms, respectively, while the detection rates in turkey birds and their environment were 95.7%, 85%, 21.3%, and 4.3%, respectively. In samples of chicken birds and their environment, carbapenem-encoding genes of *E. coli* strains (*bla*$^{KPC}$, *bla*$^{NDM}$, and *bla*$^{VIM}$) were detected in 14% (7/50), 10% (5/50), and 6% (3/50) of samples, respectively; besides, 10.6% (5/47), 10.6% (5/47), and 6.4% (3/47) of turkey bird samples were positive, respectively. These findings suggest that poultry farms, particularly turkey farms, harbor multidrug-resistant *E. coli* strains carrying clinically relevant resistance genes, posing a potential risk for zoonotic transmission and a significant public health impact.

*Prevalence of Salmonella spp. in poultry and environmental samples*

Out of 272 samples collected from chicken farms, *Salmonella* spp. were isolated from 11 samples (4%) as shown in Table 5. These isolates were recovered from cloacal swabs, as well as from meat and liver tissues. Environmental contamination in chicken farms was confined to litter, feed, and water samples, while no *Salmonella* spp. was recovered from workers' hand swabs. In contrast, a significantly higher prevalence was observed in turkey farms, with *Salmonella* spp. isolated from 31 of 170 samples, corresponding to 18.3%. These isolates were recovered from cloacal swabs, as well as from meat, liver, and heart tissues. Among environmental samples from turkey farms, *Salmonella* spp. was most frequently detected in water (37.5%), followed by litter (25%) and handlers' hand swabs (12.5%). Statistical analysis revealed no significant differences in *Salmonella* spp. prevalence among the different sample types within either poultry species ($p > 0.05$).

*Detection of ESBL and carbapenemase-encoding genes in Salmonella isolates*

An overview of the distribution of ESBL-encoding genes among various types of *Salmonella* spp. is presented in Table 5. All 11 chicken isolates were positive for *bla*$^{TEM}$ (100%), *bla*$^{SHV}$ (73%), and *bla*$^{CTX-M}$ (9%). No chicken samples contained *bla*$^{OXA-1}$. Additionally, all 31 turkey isolates tested positive for *bla*$^{TEM}$ (90%), *bla*$^{SHV}$ (74.2%), *bla*$^{CTX-M}$ (61%), and *bla*$^{OXA-1}$ (3%). Only 9% of all samples harbored carbapenem-encoding *bla*$^{NDM}$ and *bla*$^{VIM}$ genes, whereas none of the isolates harbored *bla*$^{KPC}$. In addition, only chicken meat samples contained the carbapenem-encoding *bla*$^{NDM}$ and *bla*$^{VIM}$ genes. In the turkey isolates, *bla*$^{KPC}$ and *bla*$^{NDM}$ were detected in 13% and 9.7%, respectively, in cloacal, meat, heart, and worker hand samples; however, *bla*$^{VIM}$ was not detected. These results suggest a higher frequency of both ESBL- and carbapenemase-encoding genes in *Salmonella* spp. from turkey farms, highlighting the potential zoonotic and public health risks associated with these antimicrobial-resistant strains.

*Phylogenetic analysis of bla*$^{CTX-M}$ *genes in E. coli isolates*

A phylogenetic analysis was conducted to investigate the genetic relatedness of *bla*$^{CTX-M}$ gene sequences identified in *E. coli* isolates obtained from poultry farms in Egypt (designated Zone 1 and Zone 3). Partial sequences of the *bla*$^{CTX-M}$ gene were aligned with reference sequences retrieved from GenBank, representing a range of environmental, human, and animal sources. Sequences were submitted to GenBank (Accession numbers: PV199120 and PV183229). The resulting phylogenetic tree (Fig. 2) revealed two distinct clusters corresponding to isolates from turkey (Zone 1) and chicken (Zone 3).

The Zone 1 isolate (from turkey) clustered closely with *Enterobacter* spp. and *E. coli* strains from environmental sources in India, including

**Table 3**
Total viable colony count, total coliform count, total *Enterobacterice* count, and total *Staphylococcus* count of examined samples from poultry farms (Chicken and Turkey) expressed as Mean±SE log CFU/g or ml.

| Samples | Chicken | | | | Turkey | | | |
|---|---|---|---|---|---|---|---|---|
| | TVC | TCC | TEC | TSC | TVC | TCC | TEC | TSC |
| **Meat (breast muscle)** | 3.53±0.35$^{c}$ | 2.77±0.20$^{d}$ | 2.14±0.15$^{bc}$ | 1.25±0.63 | 2.98±0.12 $^{c}$ | 2.32±0.29$^{b}$ | 1.91±0.07 | 1.25±0.63$^{b}$ |
| **Liver** | 2.47± 0.14$^{c}$ | 2.17 ± 0.30$^{d}$ | 1.84±0.07$^{c}$ | 1.90± 0.96 | 2.63 ± 0.30$^{c}$ | 2.17 ± 0.30$^{b}$ | 2.41 ± 0.08 | 1.90±0.9$^{ab}$ |
| **Cloacal swabs** | 7.14± 0.32$^{a}$ | 5.20 ± 0.10$^{b}$ | 3.12±0.07$^{a}$ | 2.51 ± 0.25 | 5.81 ± 0.09$^{a}$ | 4.00±0.85 $^{ab}$ | 2.82±0.25 | 2.51±0.25$^{a}$ |
| **Litter** | 7.86 ± 0.25$^{a}$ | 6.38± 0.09$^{a}$ | 2.41 ± 0.08$^{bc}$ | 2.82 ± 0.25 | 5.52 ± 0.38$^{a}$ | 4.48±0.37$^{a}$ | 2.74±0.41 | 2.82±0.25$^{a}$ |
| **Feed** | 5.15 ± 0.08$^{b}$ | 3.96 ± 0.17$^{c}$ | 2.54 ± 0.12$^{ab}$ | 2.63 ± 0.07 | 4.89 ± 0.36$^{ab}$ | 3.47±0.37$^{ab}$ | 2.91 ± 0.26 | 2.63±0.07$^{a}$ |
| **Water** | 3.70 ± 0.29$^{c}$ | 2.39± 0.23$^{d}$ | 2.72± 0.16$^{ab}$ | 2.28 ± 0.29 | 3.82±0.30$^{bc}$ | 2.85±0.21$^{ab}$ | 2.85±0.21 | 2.28±0.29$^{a}$ |
| **Worker' hand** | 5.67 ± 0.28$^{b}$ | 4.03± 0.11$^{c}$ | 2.82± 0.25$^{ab}$ | 2.32 ± 0.46 | 5.07 ± 0.20$^{ab}$ | 2.69±0.20$^{ab}$ | 1.48 ± 0.74 | 0.00± 0.00 |
| ***p* value** | 0.000 | 0.000 | 0.000 | 0.399 | 0.000 | 0.012 | 0.088 | 0.009 |

TVC= Total Viable Count, TCC=Total Coliform Count, TEC= Total Enterobactericie Count, TSC= Total Staphylococcus Count CFU= Colony forming unit SE= Standard error.
[a-c]Means with different lowercase letters in the same column are significantly different between different sample types ($p < 0.05$).

**Table 4**
Prevalence of *E- coli* species in poultry birds (chicken and turkey) and their related environment at the observed farms and distribution of ESBL and carbapenemase encoding genes.

| Type of Sample | Sample source | | No. Of examined samples | No. of *E- coli* positive samples (%) | ESBL encoding genes | | | | carbapenemase-encoding genes | | | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $bla_{TEM}$ | $bla_{SHV}$ | $bla_{CTX-M}$ | $bla_{OXA-1}$ | $bla_{KPC}$ | $bla_{NDM}$ | $bla_{VIM}$ | |
| Chicken | Cloacal swabs | | 44 | 23(52) | 23 | 23 | 5 | 1 | 1 | 2 | 0 | 0 .022 |
| | Organs | Meat | 60 | 9 (15) | 9 | 8 | 4 | 2 | 2 | 2 | 1 | |
| | | Liver | 60 | 3 (5) | 3 | 3 | 0 | 0 | 0 | 0 | 0 | |
| | | Heart | 60 | 3 (5) | 3 | 2 | 0 | 0 | 0 | 0 | 0 | |
| | Environmental samples* | Litter | 12 composite | 4 (33) | 4 | 4 | 1 | 1 | 1 | 1 | 0 | |
| | | Feed | 12 composite | 3 (25) | 3 | 0 | 0 | 0 | 1 | 0 | 0 | |
| | | Water | 12 composite | 3 (25) | 3 | 3 | 0 | 1 | 1 | 0 | 1 | |
| | | Worker' hand | 12 composite | 2 (17) | 2 | 2 | 1 | 0 | 1 | 0 | 1 | |
| | Total | | 272 | 50 (18.4) | 50 (100) | 45 (90) | 11(22) | 5(10) | 7(14) | 5(10) | 3(6) | |
| | *P* value | | < 0.001 | | | | | | | | | |
| Turkey | Cloacal swabs | | 18 | 8 (44) | 7 | 7 | 3 | 1 | 1 | 1 | 0 | |
| | Organs | Meat | 40 | 12 (30) | 12 | 10 | 3 | 1 | 2 | 3 | 1 | |
| | | Liver | 40 | 9 (22.5) | 9 | 9 | 2 | 0 | 0 | 0 | 1 | |
| | | Heart | 40 | 8 (20) | 7 | 7 | 1 | 0 | 0 | 0 | 0 | |
| | Environmental samples* | Litter | 8 composite | 4 (50) | 4 | 3 | 0 | 0 | 2 | 1 | 1 | |
| | | Feed | 8 composite | 4 (50) | 4 | 2 | 0 | 0 | 0 | 0 | 0 | |
| | | Water | 8 composite | 2 (25) | 2 | 2 | 1 | 0 | 0 | 0 | 0 | |
| | | Worker' hand | 8 composite | 0 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Total | | 170 | 47(27.6) | 45 (95.7) | 40 (85) | 10 (21.3) | 2 (4.3) | 5 (10.6) | 5 (10.6) | 3 (6.4) | |
| | *P* value | | 0.188 | | | | | | | | | |

*Each sample is a pool of (8-12) sample.
The result is significant at $p < 0.05$.

**Table 5**
Prevalence of Salmonella species in poultry birds (chicken and turkey) and their related environment at the observed farms and distribution of ESBL and carbapenemase encoding genes.

| Type of Sample | Source sample | | No. of examined samples | No. of salmonella positive samples | ESBL encoding genes | | | | carbapenemase-encoding genes | | | P value (chi square) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $bla_{TEM}$ | $bla_{SHV}$ | $bla_{CTX-M}$ | $bla_{OXA-1}$ | $bla_{KPC}$ | $bla_{NDM}$ | $bla_{VIM}$ | |
| Chicken | Cloacal swabs | | 44 | 1 (2.3) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | < .001 |
| | Organs | Meat | 60 | 4(6.7) | 4 | 2 | 1 | 0 | 0 | 1 | 1 | |
| | | liver | 60 | 3(5) | 3 | 3 | 0 | 0 | 0 | 0 | 0 | |
| | | heart | 60 | 0 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Environmental samples* | litter | 12 composite | 1(8.3) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | |
| | | feed | 12 composite | 1(8.3) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | water | 12 composite | 1(8.3) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | |
| | | Worker hand | 12 composite | 0(0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Total | | 272 | 11(4%) | 11 (100%) | 8 (73%) | 1(9%) | 0(0%) | 0 (0%) | 1(9%) | 1 (9%) | |
| | P value | | 0.616. | | | | | | | | | |
| Turkey | Cloacal swabs | | 18 | 6(33.3%) | 6 | 4 | 4 | 0 | 0 | 1 | 0 | |
| | Organs | Meat | 40 | 6(15%) | 6 | 6 | 5 | 1 | 2 | 1 | 0 | |
| | | Liver | 40 | 7(17.5%) | 6 | 4 | 4 | 0 | 0 | 0 | 0 | |
| | | Heart | 40 | 6(15%) | 6 | 5 | 5 | 0 | 1 | 1 | 0 | |
| | Environmental samples* | litter | 8 composite | 2(25%) | 2 | 2 | 1 | 0 | 0 | 0 | 0 | |
| | | feed | 8 composite | 0(0%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | water | 8 composite | 3(37.5%) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | |
| | | Worker hand | 8 composite | 1(12.5%) | 1 | 1 | 0 | 0 | 1 | 0 | 0 | |
| | Total | | 170 | 31(18.23) | 28(90) | 23 (74.2) | 19(61) | 1(3) | 4(13) | 3(9.7) | 0(0) | |
| | *P* value | | 0.199 | | | | | | | | | |

*Each sample is a pool of (8-12) sample.
The result is significant at $p < 0.05$.

strain HK117 and isolates from the River Yamuna. These reference strains carry *blaCTX-M-152* and *blaCTX-M-26*. Notably, the same cluster included *Klebsiella pneumoniae* isolates, suggesting possible horizontal gene transfer events among *Enterobacteriaceae* species in environmental or zoonotic settings. This genetic relatedness supports the hypothesis that the turkey isolate may have acquired its resistance gene from

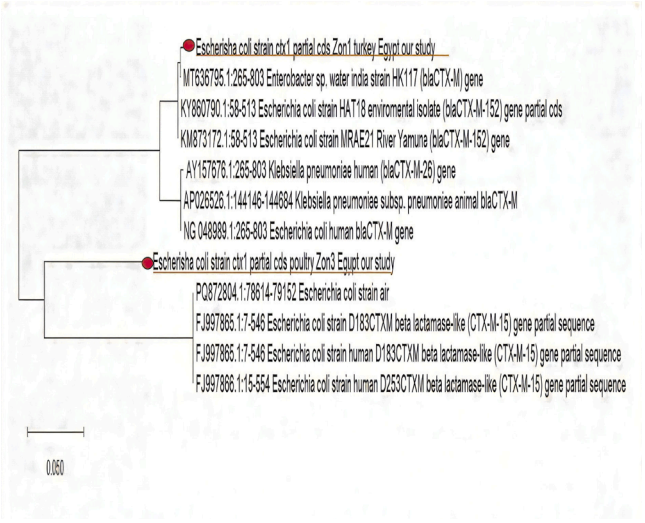


**Fig. 2.** Phylogenetic analysis of partial $bla_{CTX\text{-}M}$ gene sequences from*Escherichia coli* isolates collected from poultry farms in Egypt.

environmental or nosocomial reservoirs, thereby reinforcing the role of poultry as a potential reservoir for AMR genes.

The Zon 3 isolate (from chicken) formed a separate cluster with *E. coli* strains harboring the *blaCTX-M-15* gene, including strains D183CTXM and D253CTXM, which were previously isolated from human clinical samples and airborne sources. This genetic proximity to human- and air-derived strains suggests possible routes of transmission through environmental contamination or anthropogenic exposure. The detection of *blaCTX-M-15*, a globally disseminated and clinically important ESBL gene, raises significant public health concerns. These findings underscore the intricate ecology of resistance gene dissemination and emphasize the role of poultry in potential transmission pathways of high-risk antimicrobial resistance genes to humans and the environment.

The neighbor-joining tree was constructed using MEGA11 software based on partial sequences of $bla^{CTX\text{-}M}$ genes. Bootstrap values were calculated from 1,000 replicates and are shown at the branch points. Two isolates from this study (Zone 1 (from turkey) and Zone 3 (from chicken)) are marked with black circles. The Zone 1 isolate clustered with environmental *E. coli, Enterobacter* spp., and *Klebsiella pneumoniae* strains harboring *blaCTX-M*-152 and *blaCTX-M*-26. The Zone 3 isolate formed a distinct cluster with human and airborne*E. coli* strains carrying *blaCTX-M*-15. This clustering pattern suggests potential environmental and zoonotic dissemination of ESBL genes.

### *Antibiotic resistance profiles of E. coli and Salmonella isolates*

Table 6 presents the antimicrobial resistance profiles of Sixty-two *E. coli* and eighteen *Salmonella* isolates obtained from chicken and turkey farms, including environmental and poultry-derived samples, against multiple antibiotic classes. Among *E. coli* isolates from chicken farms, the highest resistance rates were observed against ampicillin/sulbactam (65.1%), penicillin (61.6%), and ceftriaxone (58.1%). In contrast, *E. coli* isolates from turkey farms exhibited the greatest resistance to ampicillin/sulbactam (67%), followed by penicillin (58.2%) and ceftriaxone (54.9%). Notably, isolates from both poultry species demonstrated moderate susceptibility to ofloxacin (47.3%) and clindamycin (54%), indicating that these antibiotics may still retain some efficacy. Regarding *Salmonella* isolates, a high level of resistance was observed to ceftriaxone (88.2% in chicken-derived isolates and 90.9% in turkey-derived isolates) and penicillin (82.3% and 86.3%, respectively). On the other hand, turkey-derived *Salmonella* isolates showed relatively higher sensitivity to clindamycin (50.1%) and tetracycline (41%),

**Table 6**
Percentage of antimicrobial resistance and intermediate resistance determined by disc diffusion method in *E. coli* and *Salmonella* isolates from different samples (chicken and turkey).

| Bacteria | | | | *E. coli* | | | | | | *Salmonella* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antimicrobial class | Antimicrobial agents | Disc | Conc. | resistant (%) | | intermediate (%) | | sensitive (%) | | resistant (%) | | intermediate (%) | | sensitive (%) | |
| | | | | Chicken | Turkey | Chicken | Turkey | Chicken | Turkey | Chicken | Turkey | Chicken | Turkey | Chicken | Turkey |
| flourquinolones | Ofloxacin | Ofx | 5 | 16.2 | 17.5 | 36.5 | 31.8 | 47.3 | 50.7 | 64.7 | 72.7 | 11.7 | 9 | 23.6 | 18.3 |
| Tetracycline | Tetracycline | TE | 2 | 55.8 | 52.7 | 25.5 | 24.1 | 18.7 | 23.2 | 76.4 | 59 | 0 | 0 | 23.6 | 41 |
| Cephalosporins | Ceftrixone | CRD | - | 58.1 | 54.9 | 9.3 | 10.9 | 32.6 | 34.2 | 88.2 | 90.9 | 0 | 0 | 11.8 | 9.1 |
| Lincomycin | Clindamycin | DA | 2 | 13.5 | 15.3 | 32.5 | 32.9 | 54 | 51.8 | 17.6 | 13.6 | 47 | 36.3 | 35.4 | 50.1 |
| B –lactams | Penicillin | P | | 61.6 | 58.2 | 16.2 | 20.8 | 22.2 | 21 | 82.3 | 86.3 | 0 | 0 | 17.7 | 13.7 |
| | ampicillin/sulbactam | SAM | 20 | 65.1 | 67 | 9.3 | 8.7 | 25.6 | 24.3 | 70.5 | 77.2 | 11.7 | 9 | 17.8 | 13.8 |

whereas chicken isolates were moderately sensitive to clindamycin (35.4%). These findings highlight the alarming prevalence of multidrug resistance in both *E. coli* and *Salmonella* isolates across poultry production systems, underscoring the urgent need for rational antibiotic use and the development of alternative antimicrobial strategies.

### *Heatmap analysis of antimicrobial resistance patterns in E. coli and Salmonella isolates*

As illustrated in Fig. 3, hierarchical clustering of *E. coli* isolates based on sampling source, region, and AMR profiles revealed a distinct cluster comprising isolates predominantly from chickens and turkeys in El-Menoufia Governorate. These isolates, mainly recovered from cloacal swabs and internal organs, demonstrated high resistance to penicillin, ampicillin, and ceftriaxone, indicating widespread resistance in bird-associated samples. Similarly, Fig. 4 shows the clustering pattern of *Salmonella* isolates from both chicken and turkey sources. A major cluster was formed by isolates that consistently exhibited resistance to ceftriaxone and penicillin, regardless of the poultry species. This pattern further highlights the dominance of β-lactam resistance among *Salmonella* isolates circulating within these poultry production environments. Together, these clustering analyses reinforce the link between specific AMR phenotypes and poultry-associated sampling sources, suggesting the possible role of farm-level antibiotic practices and shared environmental exposures in shaping resistance profiles.

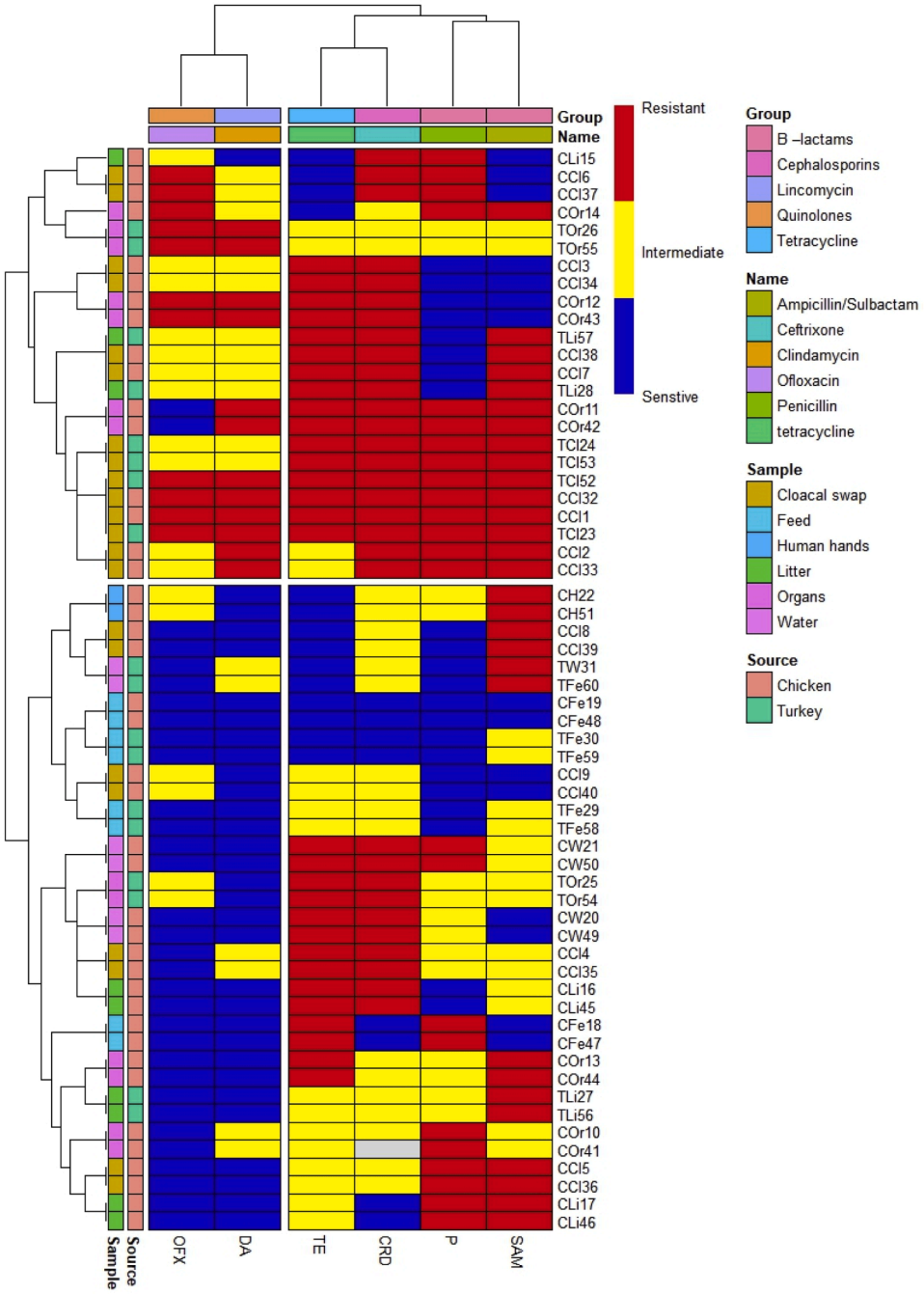


**Fig. 3.** Heatmap showing the antimicrobial resistance profiles of *E. coli* isolates ($n = 50$) from poultry farms based on sample type and location. Red indicates resistance, green indicates susceptibility. Isolates clustered by similar resistance patterns.

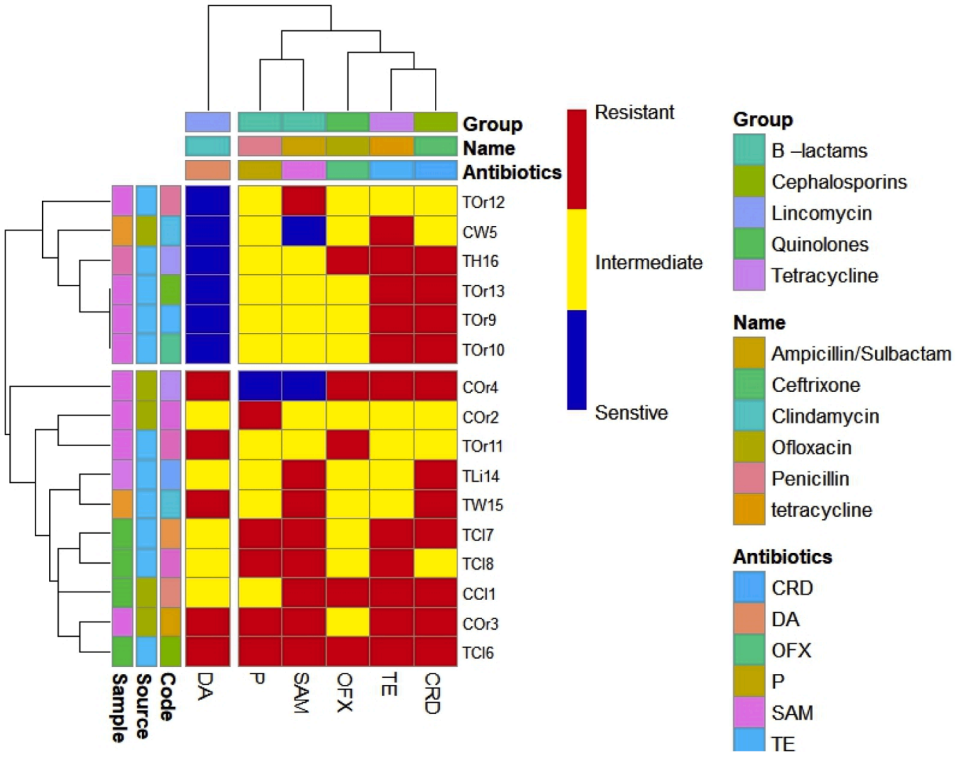


**Fig. 4.** Heatmap illustrating AMR profiles of *Salmonella* isolates ($n = 31$). Clustering based on antibiotic susceptibility to β-lactams and tetracyclines from chicken and turkey sources.

*Effect of papain treatment on bacterial counts in chicken meat during refrigerated storage*

Table 7 presents the TVC, TCC, and TEC in chicken meat samples over a 12-day refrigerated storage period. Bacterial growth was monitored in both untreated control samples and samples treated with papain. Throughout the storage period, the papain-treated group consistently exhibited lower microbial loads compared to the control. Notably, the TVC in the papain group remained significantly lower, reaching a minimum of 2.28 CFU/g on the ninth day, suggesting a substantial antibacterial effect. In contrast, the control group exhibited a progressive increase in microbial counts, with the highest TVC, TCC, and TEC levels observed by the end of the 12-day period, indicating advanced spoilage. These findings demonstrated that papain effectively delayed bacterial proliferation, thereby extending the shelf life of poultry meat under refrigeration conditions. A statistically significant difference was detected in microbial counts between treated and control samples across the storage days ($p < 0.05$).

The application of papain significantly reduced the bacterial load in chicken meat during refrigerated storage, thereby extending its shelf life. In the papain-treated group, the TVC declined from 3.55 $\log_{10}$ CFU/g on day 0 to 2.44 $\log_{10}$ CFU/g by day 12. Similarly, the TCC decreased from 2.61 $\log_{10}$ CFU/g to 1.99 $\log_{10}$ CFU/g, and the TEC dropped from 1.91 $\log_{10}$ CFU/g to 1.22 $\log_{10}$ CFU/g by the end of the storage period. Statistical analysis revealed significant differences between the treated and control groups ($p < 0.05$). Across all time points, the lowest microbial counts (TVC, TCC, and TEC) were consistently observed in the papain-treated samples, confirming its antibacterial efficacy and potential to delay spoilage during cold storage.

## Discussion

This study assessed bacterial contamination and AMR in poultry farms, with a focus on the differences between chicken and turkey production systems. The detection of multidrug-resistant *E. coli* and *Salmonella* spp. harboring ESBL- and carbapenemase-encoding genes highlights poultry as a significant reservoir for resistant pathogens with the potential to impact human health. This study, involving 12 chicken farms and eight turkey farms, revealed that chicken farms harbored a significantly higher microbial load than turkey farms. This disparity may be attributed to differences in management practices, bird physiology, housing systems, and environmental conditions (Wu et al., 2022;

**Table 7**
Total colony count, total coliform count and total *E. coli* count of examined meat and liver samples treated by (papain) expressed as a $\log_{10}$CFU/g.

| | TVC | | TCC | | TEC | |
|---|---|---|---|---|---|---|
| Days | Control | papain | Control | papain | Control | Papain |
| 0 day | 3.65±0.21$^{b}$ | 3.55±0.18$^{a}$ | 2.66±0.11d | 2.61±0.10 | 1.91±0.07$^{c}$ | 1.95±0.03$^{a}$ |
| 3rd day | 5.28±0.04$^{a}$ | 3.22±0.05$^{ab}$ | 3.16±0.03c | 2.23±0.05 | 2.55±0.22$^{b}$ | 1.59±0.0$^{b}$ |
| 6th day | S | 2.80±0.03$^{b}$ | 3.63±0.04b | 1.96±0.03 | 2.81±0.04$^{b}$ | 1.45±0.0$^{bc}$ |
| 9th day | S | 2.28±0.10$^{c}$ | 4.02±0.05a | 1.63±0.07 | 3.59±0.25$^{a}$ | 1.18±0.04$^{c}$ |
| 12th day | S | 2.44±0.07$^{c}$ | S | 1.99±0.04 | S | 1.22±0.12$^{c}$ |
| *P* value | 0.002 | 0.000 | 0.000 | 0.087 | 0.000 | 0.000 |

$^{a\text{-}c}$Means with different lowercase letters in the same column are significantly different between different sample types ($p < 0.05$).
SE=Standard error.

Shaltout et al., 2018). The highest microbial loads were recorded in litter and cloacal swabs, while liver and meat samples exhibited the lowest bacterial counts. This pattern is expected, as poultry droppings and litter often harbor a diverse population of gut-derived microorganisms (Nawar et al., 2019). Notably, high TEC values were observed in water samples and on workers' hands, emphasizing the critical role of human and environmental vectors in the transmission of bacteria within poultry production systems (Todd et al., 2008).

In turkey meat and liver samples, the TCC values were 2.14 ± 0.15 and 1.84 ± 0.07 $\log_{10}$ CFU/g, respectively, which are lower than values reported by Morshdy et al. (2023), who found 3.37 $\log_{10}$ CFU/g in poultry meat products from Zagazig City, Egypt. The lowest total *Staphylococcus* count (TSC) values in turkey were 1.25 ± 0.63 and 1.91 ± 0.07 $\log_{10}$ CFU/g in meat and liver, respectively. These findings are consistent with the results of Datta et al. (2012), who reported *Staphylococcus* spp. levels of 2.52 $\log_{10}$ CFU/g. Moreover, Jaber et al. (2017) reported even higher counts than observed in the present study. Overall, turkey farms showed consistently lower microbial loads in meat, liver, and internal organs than chicken farms, as supported by prior studies reporting lower bacterial burdens in turkeys compared to broilers at various stages of production (Jaber et al., 2017). This difference could be attributed to several factors, including smaller flock sizes, lower stocking densities, improved processing standards, or variations in feed and housing systems. Microbial contamination in poultry products is a multifaceted issue influenced by improper handling, unsanitary processing, suboptimal storage, and inadequate distribution conditions, underscoring the need for enhanced hygiene protocols. A limitation of the study is the use of conventional plate counting, which may underestimate total bacterial contamination due to its inability to detect VBNC or stressed cells. Future research could incorporate culture-independent methods (e.g., qPCR, metagenomics) to obtain a more comprehensive assessment of microbial load.

In this study, *E. coli* was detected in both chicken and turkey farm samples, with notable differences in prevalence and distribution patterns. In turkey farms, *E. coli* was detected more frequently than in chicken farms, with litter and feed serving as major environmental reservoirs. In chicken farms, *E. coli* was detected in 18.4% of samples. *E. coli* was detected in only 13% (40/304) of chicken fecal samples by Johnson et al. (2003). Similarly, Ružauskas et al. (2010) reported a higher prevalence of 41.7% in chicken liver samples, while Ahmed et al. (2025) detected *E. coli* in 51.4% of poultry internal organs. In turkey farms, *E. coli* was detected in 27.6% of samples, including cloacal swabs, meat, liver, and hearts. These findings are higher than those reported by Zhao et al. (2001), who found an *E. coli* infection rate of 11.9% in turkeys.

Regarding *Salmonella* spp., the pathogen was detected at a rate of 4% in chicken farm samples and 18.3% in turkey farm samples. These results are consistent with the findings of Mathole et al. (2017), who reported a 3.15% *Salmonella* spp. prevalence among 286 chickens in South Africa. However, they differ from more extensive surveillance data by the Center for Science in the Public Interest (CSPI), which recorded a 46.7% prevalence in broiler farms in the United States between 2019 and 2020. Furthermore, our findings contrast with those of Al-baqir et al. (2019), who reported a 32.6% isolation rate of *Salmonella* spp. from chicken farms in Egypt.

Chicken and turkey *E. coli* isolates exhibited a significant presence of ESBL-encoding genes, suggesting a role for poultry production systems in β-lactam resistance. In chicken farms, 100% of *E. coli* isolates carried the $bla^{TEM}$ gene, while $bla^{SHV}$, $blaC^{TX\text{-}M}$, and $bla^{OXA\text{-}1}$ genes were detected in 90%, 22%, and 10% of isolates, respectively. This indicates a growing prevalence of ESBL genes in food-producing animals, contributing to the global crisis of antimicrobial resistance. The predominance of $bla^{TEM}$ suggests its central role in resistance development among poultry-associated *E. coli*, while the co-occurrence of $bla^{SHV}$ and $bla^{CTX\text{-}M}$ reflects increasing genetic diversity that further complicates therapeutic management (Khalefa et al., 2025; Ahmed et al., 2025). The relatively lower detection of $bla^{OXA\text{-}1}$ indicates an ongoing evolution and diversification of resistance genes within the poultry microbiome.

Of particular concern is the identification of carbapenemase-producing *E. coli* strains. The detection of the clinically critical carbapenem-encoding genes in poultry is alarming, as carbapenems are considered last-resort antibiotics for treating multidrug-resistant bacterial infections in humans. Recent studies support the hypothesis that resistance determinants in food animals can serve as reservoirs for human infections and must be addressed through integrated One Health strategies (Liu et al., 2022). This underscores the urgent need to strengthen antimicrobial stewardship, enforce strict biosecurity protocols, and implement continuous surveillance in poultry farms to curb the dissemination of carbapenemase-producing organisms.

*Salmonella* isolates from chicken and turkey farms showed a high prevalence of ESBL-encoding genes, with $bla^{TEM}$, $bla^{SHV}$, and $bla^{CTX\text{-}M}$ genes detected in all chicken isolates. In turkey isolates, $bla^{TEM}$, $bla^{SHV}$, $bla^{CTX\text{-}M}$, and $bla^{OXA\text{-}1}$ genes were found in 61% and 3% of isolates, respectively. Moreover, $bla^{NDM}$ and $bla^{VIM}$ genes were detected in 9% of all *Salmonella* samples, signaling carbapenem resistance in zoonotic pathogens. These findings pose significant public health challenges, as the presence of *Salmonella* strains carrying carbapenemase genes may facilitate the transfer of resistance through the food chain. The growing incidence of $bla^{VIM}$ gene in animal-derived isolates is especially concerning and is consistent with reports identifying environmental reservoirs of carbapenem-resistant *Enterobacteriaceae* (Bonardi and Pitino, 2019). *E. coli* and *Salmonella* spp. have been detected in poultry, raising food safety concerns due to their antimicrobial resistance profiles. The high prevalence of ESBL-encoding genes in *E. coli* isolates indicates the widespread presence of multidrug-resistant strains. Turkey farms have a higher prevalence of *Salmonella*, highlighting the need for effective water sanitation and biosecurity measures.

*E. coli* and *Salmonella* spp. isolates from poultry and environmental sources demonstrated a concerning pattern of MDR, reflecting the broader global challenge of AMR in foodborne pathogens. Among *E. coli* isolates from chickens, high resistance rates were observed to penicillin (61.6%), ampicillin (65.1%), and ceftriaxone (58.1%), while turkey isolates showed comparable resistance to ampicillin/sulbactam (67%), penicillin (58.2%), and ceftriaxone (54.9%). Notably, resistance to third-generation cephalosporins, such as ceftriaxone, is particularly alarming, as these antibiotics are crucial for treating serious human infections (World Health Organization, 2014). The overall MDR profiles suggest excessive and possibly unregulated use of antimicrobials in poultry production, especially for prophylaxis and growth promotion. As reported by Khalefa et al. (2025), the clustering of resistant strains based on geographic origin reflects region-specific antimicrobial usage patterns and environmental factors that drive the selection and persistence of resistance. The consistent clustering of resistant isolates across commonly used antibiotics highlights the urgent need for comprehensive antimicrobial stewardship programs aimed at reducing selection pressure and limiting the spread of resistant pathogens from farm to fork.

*Salmonella* isolates from chicken and turkey exhibited high resistance to key antibiotics, including ceftriaxone and penicillin, with resistance rates of 88% and 82%, respectively. However, they showed moderate sensitivity to clindamycin and higher sensitivity to tetracycline, suggesting differences in antimicrobial exposure and farm practices. The observed resistance patterns may reflect regional antimicrobial usage and environmental pressures that select for resistant strains (Mathole et al., 2017). The clustering of *Salmonella* isolates across poultry species indicates the presence of genetically similar, resistant clones circulating within and between farms. This homogeneity in resistance profiles suggests horizontal dissemination of resistance determinants. The persistence of MDR strains in poultry necessitates a One Health approach, including strengthening antimicrobial stewardship programs, biosecurity measures, and enhanced resistance monitoring as critical steps toward mitigating the spread of MDR pathogens within the food

production chain (World Health Organization, World Organisation for Animal Health, and UN Environment, 2022; El Badawy et al., 2025).

The present study addresses critical gaps in safeguarding poultry products from microbial hazards while promoting naturally derived preservation methods by confirming the presence of pathogenic and resistant microorganisms and simultaneously investigating natural antimicrobial strategies. Papain, a cysteine protease enzyme, is widely used as a meat tenderizer, breaking down tough muscle fibers to enhance tenderness and overall quality (Zhang et al., 2025). Recognizing its safety and efficacy, the U.S. Food and Drug Administration (FDA) approved papain (EC 3.4.22.2) for artificial meat tenderization in 2019, following a thorough review of safety data (Grossman, 2019). In the present study, papain exhibited notable antimicrobial activity in poultry meat, significantly extending its shelf life. During storage, the TVC decreased from 3.55 to 2.44 $\log_{10}$ CFU/g, demonstrating its ability to suppress bacterial growth.

Papain, a proteolytic enzyme derived from papaya, disrupts bacterial cell walls, resulting in cell lysis and death. It has demonstrated minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) against various bacteria, thereby showcasing its broad-spectrum antimicrobial property (Gartika et al., 2019). The application of papain in meat products has been shown to significantly reduce microbial proliferation, thereby prolonging the shelf life. Verma et al. (2022). Found that meat emulsions treated with papain exhibited lower microbial counts than untreated controls, enhancing product safety and quality. While papain is effective, other natural preservatives, such as bacteriocins, also show promise in extending the shelf life of poultry meat, suggesting the potential for their combined use in food preservation strategies (Umiralieva et al., 2025). Additionally, reductions in coliform and *Enterobacteriaceae* counts reflected papain's broad-spectrum antibacterial action against common spoilage organisms and potential foodborne pathogens. Statistically significant differences ($p < 0.05$) between treated and control groups further validate its effectiveness as a natural preservative. These results align with previous findings, such as those by Olaniyi (2018), who reported that natural plant-derived extracts like ginger reduce microbial loads and prolong poultry meat shelf life.

## Conclusion

This study highlights the challenge of bacterial contamination and AMR in both chicken and turkey farms. A high prevalence of MDR pathogens, particularly *Escherichia coli* and *Salmonella* spp., was detected, with widespread resistance to commonly used antibiotics, including ampicillin, tetracycline, and sulfonamides. Antimicrobial use in poultry production, especially in high-density farming systems, remains a key driver of AMR. Current antimicrobial practices are strongly associated with elevated resistance rates compared to historical patterns. Poultry farms serve as potential reservoirs for the dissemination of resistant bacteria to humans, other animals, and the environment through direct contact and environmental contamination. Despite some progress in limiting antibiotic use, AMR persists as a serious public health threat. To address this issue effectively, integrated interventions are urgently needed. These include strict biosecurity protocols, responsible antimicrobial stewardship, and continuous monitoring and surveillance programs. Adopting a One Health approach that links animal, human, and environmental health is crucial for curbing the emergence and spread of AMR in poultry production systems. This is essential to safeguarding public health. Moreover, papain's promising results suggest its potential as a natural, safe alternative to synthetic preservatives. This aligns with consumer preferences for minimally processed, chemical-free foods. Future research should focus on gaining mechanistic insights into papain's antimicrobial activity, determining optimal application doses, and evaluating its efficacy against a broader spectrum of pathogens under various storage conditions.

## Funding

Not applicable.

## Data availability

Data available in manuscript.

## Ethical approval

The protocol was conducted according to ethical guidelines of the Institutional Animal Use and Care Committee (Vet. CU. IACUC), for the use of laboratory animals with the approval code Vet CU110520251178.

## Funding

Not applicable.

## CRediT authorship contribution statement

**Hanan S. Khalefa:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Zeinab S. Ahmed:** Writing – original draft, Methodology, Data curation. **Amr Abd El-khalik A. El-Saadany:** Writing – original draft, Resources, Data curation. **Dina Ismail Elzahaby:** Resources, Project administration, Formal analysis. **Mona M. Abd El-Fattah:** Resources, Methodology, Investigation. **Enas A. Shedeed:** Methodology, Funding acquisition, Formal analysis. **Nesma M. Kamel:** Software, Resources, Funding acquisition, Formal analysis. **Eman.F.E. Shebl:** Investigation, Funding acquisition, Formal analysis. **May F. Abdelaty:** Resources, Project administration, Investigation, Formal analysis. **Amani Abd El Latif Mosleh:** Writing – original draft, Visualization, Methodology, Funding acquisition.

## Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

Not applicable.

## References

Ahmed, A.A., Salem, H.M., Hamoud, M.M., Amer, M.M., 2025. Avian colibacillosis, multidrug resistance, antibiotic alternatives: an updated review. Egypt. J. Vet. Sci. 1–21.

Ahmed, Z.S., Hashad, M.E., Atef, Y., Badr, H., Elhariri, M., Kadry, M., 2025. Public health threat of antimicrobial resistance and virulence genes in Escherichia coli from human-chicken transmission in Egypt. Sci. Rep. 15 (1), 12627.

Al-baqir, A., Hussein, A., Ghanem, I., Megahed, M., 2019. Characterization of paratyphoid salmonellae isolated from broiler chickens at Sharkia governorate, Egypt. Zagazig Vet. J. 47 (2), 183–192.

Alam, S.B., Mahmud, M., Akter, R., Hasan, M., Sobur, A., Nazir, K.N.H., Rahman, M., 2020. Molecular detection of multidrug resistant Salmonella species isolated from broiler farm in Bangladesh. Pathogens 9 (3), 201.

American Public Health Association (APHA), 2001. Compendium of Methods for the Microbiological Examination of Food, 4th ed. American Public Health Association, Washington, D.C.

Andino, A., Hanning, I., 2015. Salmonella enterica: survival, colonization, and virulence differences among serovars. Sci. World J. 2015 (1), 520179.

Aslam, M., Checkley, S., Avery, B., Chalmers, G., Bohaychuk, V., Gensler, G., Boerlin, P., 2012. Phenotypic and genetic characterization of antimicrobial resistance in Salmonella serovars isolated from retail meats in Alberta, Canada. Food Microbiol. 32 (1), 110–117.

Bonardi, S., Pitino, R., 2019. Carbapenemase-producing bacteria in food-producing animals, wildlife and environment: a challenge for human health. Ital. J. Food Saf. 8 (2), 7956.

CLSI Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI Doc.M100 30th edn. (CLSI, 2020).

Codjoe, F.S., Donkor, E.S., 2017. Carbapenem resistance: a review. Med. Sci. 6 (1), 1.

Dadgostar, P., 2019. Antimicrobial resistance: implications and costs. Infect. Drug Resist. 3903–3910.
Danon-Moshe, S., Kozareva, M.N., Paparikova, K.D., 1985. Sanitary microbiology–methods of study. Med. Phizkultura Sofia 95–109.
Datta, S., Akter, A., Shah, I.G., Fatema, K., Islam, T.H., Bandyopadhyay, A., Biswas, D., 2012. Microbiological quality assessment of raw meat and meat products, and antibiotic susceptibility of isolated Staphylococcus aureus. Agric. Food Anal. Bacteriol. 2 (3), 187–194.
Dec, M., Nowaczek, A., Stępień-Pyśniak, D., Wawrzykowski, J., Urban-Chmiel, R., 2018. Identification and antibiotic susceptibility of lactobacilli isolated from turkeys. BMC Microbiol. 18 (1), 168.
El Badawy, A., Hamza, D., Ahmed, Z., Sabry, M.A., 2025. Seasonal and environmental drivers of antibiotic resistance and virulence in Escherichia coli from aquaculture and their public health implications. Sci. Rep. 15 (1), 16100.
Elkholy, R.A.A.M., Hussein, M.N.A., Abou EL-Roos, N., Shaltout, F., 2024. Enhancing microbiological and histological quality of frozen Turkey meat using vinegar. Egypt. J. Vet. Sci. 1–8.
Eshamah, H., Han, I., Naas, H., Acton, J., Dawson, P., 2014. Antibacterial effects of natural tenderizing enzymes on different strains of Escherichia coli O157: H7 and Listeria monocytogenes on beef. Meat Sci. 96 (4), 1494–1500.
Gartika, M., Satari, M.H., Chairulfattah, A., Hilmanto, D., 2019. The effect of papain towards mRNA expression of gtfB, gtfC, gtfD, gbpB and Streptococcus mutans biofilm mass formation. J. Int. Dent. Med. Res. 12 (4), 1335–1342.
Grossman, M.R., 2019. USDA and FDA formal agreement on regulation of cultured meat. Eur. Food Feed Law Rev. 14 (4), 385–389.
Guabiraba, R., Schouler, C., 2015. Avian colibacillosis: still many black holes. FEMS Microbiol. Lett. 362 (15), fnv118.
Gyawali, R., Ibrahim, S.A., 2014. Natural products as antimicrobial agents. Food Control 46, 412–429.
Ibrahim, A.N., Khalefa, H.S., Mubarak, S.T., 2023. Residual contamination and biofilm formation by opportunistic pathogens Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa in poultry houses isolated from drinking water systems, fans, and floors. Egypt. J. Vet. Sci. 54 (6), 1041–1057.
Ismael, E., Ismail, E. M., Khalefa, H. S., Elleithy, E. M., Elmosalamy, S. H., Marouf, S., & Fahmy, K. N. E. D. (2022). Evaluation of Saccharomyces cerevisiae yeast fermentate and xylanase in reduced energy diet fed to broiler chicken.
ISO "International Standards Organization" (4833-1), 2013. Microbiology of Food Chain-Horizontal Method for the Enumeration of Microorganisms. Part I; Colony Count at 30 °C by the Pour Plate Technique. International Standards Organization, Geneva, Switzerland.
Jaber, H., Ijoub, R., Zaher, A., Chakit, M., Rhaiem, N., Bourkhiss, B., Ouhssine, M., 2017. Microbiological study of turkey meat marketed in Kenitra (North-oust of Morocco). J. Nutr. Food Sci. 7 (04).
Johnson, J.R., Murray, A.C., Gajewski, A., Sullivan, M., Snippes, P., Kuskowski, M.A., Smith, K.E., 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic Escherichia coli from retail chicken products. Antimicrob. Agents Chemother 47 (7), 2161–2168.
Kadry, M., Nader, S.M., Elshafiee, E.A., Ahmed, Z.S., 2021. Molecular characterization of ESBL and carbapenenemase producing Salmonella spp. Isolated from chicken and its public health importance. Pak. J. Zool. 53 (6).
Kagirita, A.A., Baguma, A., Owalla, T.J., Bazira, J., Majalija, S., 2017. Molecular characterization of Salmonella from human and animal origins in Uganda. Int. J. Bacteriol. 2017 (1), 4604789.
Karkal, S.S., Venmarath, A., Velappan, S.P., Kudre, T.G., 2022. Enzymes in meat, fish, and poultry product processing and preservation-II. Novel Food Grade Enzymes: Applications in Food Processing and Preservation Industries. Springer Nature Singapore, Singapore, pp. 193–216.
Khalefa, H.S., Ahmed, Z.S., Abdel-Kader, F., Ismail, E.M., Elshafiee, E.A., 2021. Sequencing and phylogenetic analysis of the stn gene of Salmonella species isolated from different environmental sources at Lake Qarun protectorate: the role of migratory birds and public health importance. Vet. World 14 (10), 2764.
Khalefa, H.S., Arafa, A.A., Hamza, D., El-Razik, K.A.A., Ahmed, Z., 2025. Emerging biofilm formation and disinfectant susceptibility of ESBL-producing Klebsiella pneumoniae. Sci. Rep. 15 (1), 1599.
Kolde, R. Pheatmap: pretty heatmaps. R package version 1.0. 12. https://CRAN.R-project.org/package=;pheatmap (2019).
Liu, X., Li, R., Chan, E.W.C., Xia, X., Chen, S., 2022. Plasmid-mediated ciprofloxacin, carbapenem and colistin resistance of a foodborne Escherichia coli isolate. Food Control 137, 108937.
Llerena-Suster, C.R., Priolo, N.S., Morcelle, S.R., 2011. Sodium tetrathionate effect on papain purification from different Carica papaya latex crude extracts. Prep. Biochem. Biotechnol. 41 (2), 107–121.
Mak, P.H., Rehman, M.A., Kiarie, E.G., Topp, E., Diarra, M.S., 2022. Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review. J. Anim. Sci. Biotechnol. 13 (1), 148.
Mathole, M.A., Muchadeyi, F.C., Mdladla, K., Malatji, D.P., Dzomba, E.F., Madoroba, E., 2017. Presence, distribution, serotypes and antimicrobial resistance profiles of Salmonella among pigs, chickens and goats in South Africa. Food Control 72, 219–224.
Moawad, A.A., Hotzel, H., Hafez, H.M., Ramadan, H., Tomaso, H., Braun, S.D., Monecke, S., 2022. Occurrence, phenotypic and molecular characteristics of extended-spectrum beta-lactamase-producing Escherichia coli in healthy turkeys in northern Egypt. Antibiotics 11 (8), 1075.
Morshdy, A.E.M., Darwish, W., Mohammed, F.M., Mahmoud, A.F.A., 2023. Bacteriological quality of retailed chicken meat products in Zagazig City, Egypt. J. Adv. Vet. Res. 13 (1), 47–51.
Morshdy, A.E., Eltahlawy, A.S.M.A., Sakr, N.M., Eissa, K., Elshopary, N.F., El-Naby, G.R., Elbayomi, R.M., 2025. Comparative study on the effect of Carica Papaya and Moringa oleifera extracts on Aspergillus fungi growth in minced meat under refrigerated storage. Egypt. J. Vet. Sci. 56 (13), 89–98.
Mughini-Gras, L., Dorado-García, A., van Duijkeren, E., van den Bunt, G., Dierikx, C.M., Bonten, M.J., Heederik, D.J., 2019. Attributable sources of community-acquired carriage of Escherichia coli containing β-lactam antibiotic resistance genes: a population-based modelling study. Lancet Planet. Health 3 (8), e357–e369.
Murugkar, H.V., Rahman, H., Dutta, P.K., 2003. Distribution of virulence genes in Salmonella serovars isolated from man & animals. Indian J. Med. Res. 117, 66.
Nawar, Z. M., Ahmed, S. A., El-Iraqi, G. K., Ismail, E. M., Khalefa, H. S., .& El-Mosalamy, S. H. (.2019). Impacts of corncobs as an alternative litter material on broiler welfare reared in deep litter system.
Olaniyi, O.O., 2018. Microbiological, nutritional quality and antioxidant activity of fermented Delonix regia seeds. J. Food Saf. Hyg. 4 (3/4).
Olasz, A., Dobra, P. F., Kerek, A., jerzsele, A., & Baita, L. (2023). In vivo efficacy of different extracts of propolis in broiler salmonellosis.
Pitout, J.D., 2010. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs 70 (3), 313–333.
Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., Fitzpatrick, E., 2011. Veterinary Microbiology and Microbial Disease. John Wiley & Sons.
Ružauskas, M., Šiugždinienė, R., Krikštolaitis, R., Virgailis, M., Zienius, D., 2010. Prevalence and antimicrobial resistance of E. coli isolated from chicken liver sold in retail markets. Vet. Med. Zoot. 52 (74), 67–72.
Samir, A., Abdel-Moein, K.A., Zaher, H.M., 2022. The public health burden of virulent extended-spectrum β-lactamase-producing Klebsiella pneumoniae strains isolated from diseased horses. Vector-Borne Zoonotic Dis. 22 (4), 217–224.
Shakal, M., Khalefa, H.S., Salem, H., 2024. Antibacterial efficacy of Zinc oxide nanoparticles against Escherichia coli experimental infection in broiler chickens. J. Adv. Vet. Res. 14 (4), 687–691.
Shalaby, A., Ismail, M.M., El-Sharkawy, H., 2022. Isolation, identification, and genetic characterization of antibiotic resistance of Salmonella species isolated from chicken farms. J. Trop. Med. 2022 (1), 6065831.
Shaltout, F.A., El-Shorah, H.F., El Zahaby, D.I., Lotfy, L.M., 2018. Bacteriological profile of chicken meat products. Food Nutr Current Res 1 (3), 83–90.
Shoukry, M.M., 2021. The future of livestock development in Egypt from perspective of current and future challenges. Egypt. J. Nutr. Feeds 24 (2), 1–7.
Tariq, S., Samad, A., Hamza, M., Ahmer, A., Muazzam, A., Ahmad, S., Amhabj, A.M.A., 2022. Salmonella in poultry; an overview. Int. J. Multidiscip. Sci. Arts 1 (1), 80–84.
Todd, E.C., Greig, J.D., Bartleson, C.A., Michaels, B.S., 2008. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 5. Sources of contamination and pathogen excretion from infected persons. J. Food Prot. 71 (12), 2582–2629.
Umiralieva, L.B., Ospanov, A.B., Ismailova, E.T., Filatov, I.D., 2025. Analytical review of microbiological aspects of production of biological products of starters for baking pastry based on highly active cultures of microorganisms. Bull. Shakarim Univ., Tech. Sci. 3 (19), 390–407.
Verma, A.K., Chatli, M.K., Mehta, N., Kumar, P., 2022. Antimicrobial and antioxidant potential of papain liver hydrolysate in meat emulsion model at chilling storage under aerobic packaging condition. Waste BioMass Valorization 13 (1), 417–429.
Wang, G., Clark, C.G., Rodgers, F.G., 2002. Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the O157: H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J. Clin. Microbiol. 40 (10), 3613–3619.
World Health Organization. (2014). Antimicrobial resistance global report on surveillance: 2014 summary. In Antimicrobial Resistance Global Report on Surveillance: 2014 Summary.
World Health Organization, World Organisation for Animal Health, & UN Environment, 2022. Antimicrobial Resistance Multi-Partner Trust Fund Annual Report 2021: Annual Report 2023. World Health Organization.
Wu, X., Suksawat, F., Angkititrakul, S., Song, C., Chai, J., Zhang, Y., Li, W., 2022. A systematic review: the source of antimicrobial resistance pathogenic bacteria on slaughtered meat, cross-contamination or self-contamination? Thai J. Vet. Med. 52 (3), 423–440.
Youseef, M., Karam, F., Kadry, M., Elhariri, M., Elhelw, R., 2024. Escherichia coli and their potential transmission of carbapenem and colistin-resistant genes in camels. BMC Microbiol. 24 (1), 65.
Zhang, Z., Wu, Y., Zhang, C., Huang, F., 2025. Exploring how papaya juice improves meat tenderness and digestive characteristics in Wenchang chickens. Poult. Sci. 104 (1), 104621.
Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., Meng, J., 2001. Prevalence of Campylobacter spp., Escherichia coli, and Salmonella serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. Appl. Environ. Microbiol. 67 (12), 5431–5436.