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Prevalence and Antimicrobial Resistance of *Escherichia coli* Isolated from Table Eggs in Kabul City, Afghanistan

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ABSTRACT

A cross-sectional study was carried out from January to March 2025 to assess the prevalence of *Escherichia coli* contamination in table eggs sold throughout Kabul and to characterize the antimicrobial resistance (AMR) profiles of the isolated strains. A total of 150 eggs were collected systematically from various retail outlets, including supermarkets and local markets. To determine the contamination source, eggshells and internal contents were cultured separately. Presumptive *E. coli* colonies were confirmed using standard biochemical tests. Antimicrobial susceptibility was subsequently evaluated using the Kirby-Bauer disk diffusion method against a panel of six clinically relevant antibiotics. The findings revealed that *E. coli* was present on 43.3% of eggshells and in 18.0% of egg contents, suggesting potential failures in handling and storage practices. Notably, contamination rates were significantly higher in eggs from local production systems than in those from commercial sources. Antimicrobial susceptibility testing demonstrated high levels of resistance to commonly used antibiotics, including tetracycline (76.9%), ampicillin (69.2%), and sulfamethoxazole (61.5%). Critically, multidrug resistance (MDR), defined as resistance to three or more drug classes, was observed in 57.7% of all isolates, with a significantly higher rate in isolates from local eggs (68.4%). This study reveals a high prevalence of *E. coli* contamination in eggs sold in Kabul, with the isolates displaying alarming levels of AMR and MDR. The findings underscore an urgent need to implement improved hygiene practices across the entire egg supply chain and to strengthen antimicrobial stewardship policies to safeguard public health in Afghanistan.

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INTRODUCTION

Eggs are among the most widely consumed animal-derived foods worldwide due to their affordability, nutritional richness, and culinary versatility. They are a staple food in both developed and developing countries and serve as an essential dietary component for diverse populations regardless of cultural or socioeconomic background. Eggs are widely recognized as a highly bioavailable source of animal protein, providing all essential amino acids in balanced proportions, thereby qualifying as a complete protein source for human nutrition (Miranda et al., 2015). Beyond their protein content, eggs are rich in a variety of

micronutrients essential for growth, development, and health maintenance. These include fat-soluble vitamins (A, D, E, and K); water-soluble vitamins (B12, riboflavin, and folate); and minerals (selenium, phosphorus, zinc, and iron). Collectively, these nutrients contribute to metabolic regulation, immune competence, cognitive development, and reproductive health. Certain enriched egg varieties, such as those fortified with omega-3 fatty acids, have been linked to cardiovascular benefits and improved neurological function. Owing to these attributes, eggs are often regarded as a functional food capable of addressing multiple nutritional needs simultaneously (Réhault-Godbert et al., 2019).

In addition to their nutritional significance, eggs have considerable economic and cultural importance. They are inexpensive relative to other sources of animal protein, making them accessible to large segments of the population in low- and middle-income countries (LMICs). They are also widely used in culinary practices, from household meals to commercial food processing, further underscoring their role as a versatile and indispensable food commodity. In countries like Afghanistan, eggs are not only consumed as a daily dietary item but also provide an essential source of livelihood for thousands of smallholder farmers who raise poultry for both subsistence and local markets. Thus, eggs contribute to household food security, income generation, and women's economic participation, as backyard poultry keeping is often managed by women in rural settings (Wong et al., 2017; Zahir et al., 2024).

Despite these nutritional and socioeconomic benefits, eggs are highly vulnerable to microbial contamination when biosecurity, hygienic handling, and storage practices are not adequately maintained. Foodborne pathogens are well documented as causes of egg-associated infections, with Escherichia coli (E. coli) a major concern. E. coli is a gram-negative, facultatively anaerobic bacterium that naturally inhabits the intestinal tract of humans and animals (Cheesbrough, 2006). While commensal strains of E. coli are harmless, pathogenic variants have been implicated in both intestinal and extraintestinal infections in humans, ranging from gastroenteritis and hemorrhagic colitis to urinary tract infections and septicemia. Importantly, the presence of E. coli in eggs is not merely an indicator of bacterial contamination but is also considered a sentinel for fecal pollution, reflecting hygiene lapses at farm, collection, or retail levels (Taskeen et al., 2025).

Egg contamination with E. coli may occur through two principal mechanisms: vertical and horizontal transmission. Vertical transmission occurs when bacteria colonize the hen's reproductive tract and infect the egg during formation, before the protective shell has been deposited. In this case, contamination originates internally and may persist despite surface cleaning or disinfection efforts. Horizontal transmission, on the other hand, takes place post-laying and is commonly associated with environmental exposure. Contact with contaminated fecal matter, dirty nesting materials, inadequately cleaned equipment, or human handling are key risk factors (Dognon et al., 2026; Dushayeva, 2025). Retail-level practices, including lack of refrigeration, bulk selling of unwashed eggs, and exposure to high ambient temperatures, further contribute to microbial proliferation. The persistence and growth of bacteria on eggs are also influenced by external factors, such as relative humidity,

seasonality, and handling methods, which play a critical role in determining the microbial load and overall food safety risk.

Research worldwide has shown that eggs from informal or backyard production systems tend to have higher contamination levels than those from industrial, regulated operations. Backyard poultry systems often lack standardized biosecurity measures, including routine cleaning, vaccination programs, and monitoring of feed and water quality. In such settings, poultry are commonly reared in close contact with other domestic animals or humans, which increases the likelihood of pathogen transmission. Conversely, regulated industrial farms generally follow stricter sanitary practices such as automated egg collection, washing, grading, and controlled cold-chain storage, which reduce but do not eliminate the risk of contamination (Taskeen et al., 2025; von Kiparski et al., 2025). Importantly, even in highly controlled systems, breaches in protocols or improper cold chain maintenance during transportation and marketing can compromise egg safety.

Beyond contamination alone, the growing problem of antimicrobial resistance (AMR) among foodborne pathogens, including E. coli, poses a substantial threat to global health. The World Health Organization (WHO, 2017) has recognized AMR as one of the ten most significant global health challenges of the 21st century. The emergence of resistant strains has been strongly linked to the overuse and misuse of antimicrobials in both human medicine and animal agriculture. In the poultry industry, antibiotics are widely used not only for therapeutic interventions but also as prophylactic agents and growth promoters, practices that exert selective pressure on bacterial populations and favor the proliferation of resistant strains (Dushayeva, 2025; Oladipo et al., 2026).

Multidrug-resistant (MDR) E. coli strains represent a dual hazard: first, they may cause infections in humans that are difficult or impossible to treat with conventional antibiotics; second, they serve as reservoirs of resistance genes that can be transferred horizontally to other bacteria via mobile genetic elements such as plasmids and transposons (Tola et al., 2026). This gene transfer potential accelerates the spread of resistance within bacterial communities, amplifying the threat across the food chain. Evidence from South Asia and the Middle East has already revealed alarming levels of resistance among E. coli isolates from poultry products to widely used antibiotics such as ampicillin, tetracycline, and sulfamethoxazole (Taskeen et al., 2025). More concerning, resistance is emerging against critically important antimicrobials for human health, including fluoroquinolones and third-generation cephalosporins. These trends reduce therapeutic options for clinical treatment, increase the probability of treatment failure, and raise overall morbidity and mortality from bacterial infections.

In LMICs, the risk of AMR is compounded by weak regulatory frameworks, limited veterinary services, limited producer awareness, and insufficient diagnostic capacity (FAO, 2020). Antibiotics are often readily available in local markets without a prescription, leading to indiscriminate and frequent use in poultry production. Withdrawal periods before marketing eggs or poultry meat are rarely observed, resulting in residues in food products

and further promoting the development of resistant bacterial strains. Moreover, national surveillance systems for monitoring antimicrobial use and resistance remain poorly developed, creating critical knowledge gaps for risk assessment and policymaking (FAO, 2020; Dushayeva, 2025).

Afghanistan exemplifies many of these challenges. Poultry farming in the country is predominantly small-scale and informal, with most eggs being marketed through street vendors and open-air markets without packaging, refrigeration, or sanitary oversight. The absence of standardized grading and egg-washing practices, coupled with minimal enforcement of food safety guidelines, creates ideal conditions for microbial contamination. Furthermore, the lack of veterinary public health infrastructure, combined with unregulated access to antibiotics, increases the likelihood of AMR emergence and dissemination in the food chain. Published data on the prevalence of microbial contamination and resistance profiles in Afghan poultry products are extremely limited, which hampers evidence-based policymaking and the development of intervention strategies (FAO, 2023; Saudi Food and Drug Authority, 2026).

From a public health perspective, the risks are profound. Eggs are consumed by nearly all population groups, including children, pregnant women, and immunocompromised individuals, who may be particularly vulnerable to foodborne infections. The presence of MDR E. coli in eggs could therefore have far-reaching consequences, contributing not only to localized foodborne outbreaks but also to the broader dissemination of resistant strains within communities. The potential economic burden includes increased healthcare costs due to prolonged illnesses, reduced productivity, and reduced consumer confidence in poultry products, which may, in turn, undermine food security and livelihoods (Oladipo et al., 2026).

Given these concerns, research into egg contamination and resistance patterns is both timely and necessary. While numerous studies have been conducted in other regions of the world, Afghanistan remains underrepresented in the scientific literature. Establishing baseline data is essential to understanding the extent of the problem and developing locally tailored strategies for risk mitigation. The present cross-sectional study was designed to achieve the following primary objectives:

- To determine the overall prevalence of Escherichia coli (E. coli) contamination in table eggs sold across diverse retail settings in Kabul city.
- To conduct a comparative analysis of E. coli contamination rates between eggs originating from local (backyard) production systems and those from industrial (commercial) production systems.
- To characterize the antimicrobial resistance profiles of the recovered E. coli isolates against a panel of commonly used antibiotics.
- To evaluate the prevalence of multidrug-resistant (MDR) E. coli strains among the positive isolates obtained from the egg samples.

MATERIALS AND METHODS

This cross-sectional study was conducted in Kabul, the capital city of Afghanistan, between January and March 2025. The study period was strategically chosen to avoid seasonal temperature extremes, as both high summer heat and cold winter conditions can influence bacterial survival and proliferation during transport and storage, potentially biasing prevalence estimates. By focusing on this moderate temperature period, the study aimed to capture contamination levels that reflect typical consumer exposure under relatively stable environmental conditions.

Sample Size and Collection Strategy

In this study, 150 table eggs were collected using a stratified random sampling approach to ensure adequate representation of the diverse retail environments in Kabul. Five major retail sources were selected to reflect the primary distribution channels through which consumers purchase eggs. These included street vendors (n = 30), open-air markets (n = 60) sampled from two distinct market locations, and supermarkets (n = 60) obtained from two different supermarket chains. This sampling framework was designed to capture potential variations in handling practices, storage conditions, and hygiene standards across different retail settings.

At each retail source, eggs were sampled randomly from available lots. All eggs were visibly clean and free from cracks to simulate typical consumer purchasing conditions. Upon collection, eggs were immediately placed in sterile, insulated containers maintained at approximately 4°C and transported to the microbiology laboratory at Kabul University within four hours of collection. Each egg was assigned a unique identification code documenting retail source, date of collection, and production type classification.

Classification by Production System

Based on source verification and packaging information, the eggs were categorized into two distinct production systems. A total of 75 samples were classified as local eggs, originating from small-scale or backyard poultry systems. These eggs were typically sold loose, unwashed, and without refrigeration. The birds producing these eggs are generally raised in open environments with minimal biosecurity measures, which may increase the risk of contamination.

The remaining 75 samples were classified as industrial eggs, sourced from commercial poultry farms operating under regulated management systems. These systems include controlled housing conditions, routine sanitation practices, and maintenance of the cold chain during storage and distribution. Industrial eggs were typically packaged and properly labeled, reflecting standardized production and handling procedures.

Sample Processing and Microbiological Analysis

All laboratory procedures were conducted under strict aseptic conditions to prevent cross-contamination. For eggshell sampling, sterile cotton swabs pre-moistened with buffered

peptone water (BPW) were rubbed over the entire surface of each egg using a standardized zigzag pattern. The swabs were then transferred into tubes containing 10 mL of BPW for pre-enrichment. For egg content sampling, the shell surface was first disinfected with 70% ethanol. Each egg was aseptically cracked using a sterile blade, and the entire contents, including albumen and yolk, were collected into sterile containers. The contents were homogenized by gentle vortexing, and 25 mL of the homogenate was transferred into 225 mL of BPW for pre-enrichment.

All pre-enrichment cultures were incubated at 37°C for 18–24 hours. After incubation, 0.1 mL aliquots from each enriched sample were streaked onto MacConkey agar to assess lactose fermentation and onto Eosin Methylene Blue (EMB) agar to observe the characteristic metallic green sheen associated with *Escherichia coli*. The inoculated plates were incubated at 37°C for 24 hours.

Biochemical Confirmation

Suspected colonies showing lactose-positive reactions on MacConkey agar and a metallic sheen on EMB agar were subcultured on nutrient agar for purification. Pure isolates were then subjected to a standard panel of biochemical tests for confirmation of E. coli, including indole production (positive), methyl red test (positive), Voges–Proskauer test (negative), citrate utilization test (negative), oxidase test (negative), and catalase test (positive). Presumptive isolates confirmed by these biochemical characteristics were stored in tryptic soy broth supplemented with 20% glycerol at −20°C for subsequent antimicrobial susceptibility testing.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Seven antimicrobial agents were selected based on their relevance to human clinical therapy, common use in poultry production in Afghanistan, and inclusion in global antimicrobial resistance surveillance programs. These included ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), and cefotaxime (30 µg), the latter representing a third-generation cephalosporin of critical importance in human medicine. For each isolate, a bacterial suspension equivalent to a 0.5 McFarland turbidity standard was prepared in sterile saline. Mueller–Hinton agar plates were inoculated using sterile cotton swabs to ensure confluent bacterial growth. Antibiotic disks were applied to the agar surface using sterile forceps with adequate spacing to avoid overlapping inhibition zones. The plates were incubated at 37°C for 18–24 hours.

Inhibition zone diameters were measured using a digital caliper and interpreted according to CLSI 2023 breakpoints for Enterobacteriaceae. Isolates were categorized as susceptible, intermediate, or resistant. For analytical purposes, intermediate isolates were classified as resistant to provide a conservative estimate of clinically significant resistance.

Definition of Multidrug Resistance

Multidrug resistance was defined as acquired resistance to at least one antimicrobial agent in three or more antimicrobial categories, in accordance with established epidemiological criteria (Magiorakos et al., 2012).

Quality Control

hroughout the study period, *E. coli* ATCC 25922 was used as a reference strain for quality control in both biochemical identification and antimicrobial susceptibility testing. The sterility of all culture media was verified by incubating uninoculated plates and broths to ensure the absence of contamination.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY). To determine the overall burden of contamination and the extent of antimicrobial resistance, descriptive statistics were used to calculate the prevalence of E. coli (expressed as percentages) and the resistance rates to each tested antibiotic.

To investigate potential risk factors and sources of contamination, comparative analyses were performed. The chi-square test of independence was employed to compare categorical outcomes between independent groups. This test was specifically chosen to determine if there was a statistically significant association between the type of production system (local vs. industrial) and the prevalence of E. coli contamination. The same test was applied to compare contamination rates between eggshells and internal contents. When any expected cell frequency in the contingency table was less than 5, Fisher's exact test was used instead, as it provides a more accurate p-value for small sample sizes. For all statistical tests, a p-value of less than 0.05 was considered the threshold for statistical significance, indicating that the observed differences were unlikely to have occurred by chance alone.

FINDINGS

Of the 150 table eggs analyzed, *Escherichia coli* was isolated from 65 eggshell samples (43.3%) and 27 egg content samples (18.0%). These findings indicate substantial microbial contamination of eggs sold in Kabul, with eggshell contamination occurring more than twice as frequently as internal contamination. The overall prevalence of 43.3% on eggshells and 18.0% in egg contents confirms that table eggs sold in Kabul retail settings represent a potential vehicle for foodborne exposure to *E. coli*.

When analyzed by production system, local (backyard) eggs showed significantly higher contamination levels than industrial (commercial) eggs. As shown in Table 1, *E. coli* was recovered from 39 of 75 local eggshells (52.0%) compared to 26 of 75 industrial eggshells (34.7%) ($p = 0.032$). Similarly, contamination of egg contents was detected in 18 of 75 local eggs (24.0%) versus 9 of 75 industrial eggs (12.0%) ($p = 0.045$). These differences underscore the influence of production practices and biosecurity measures on egg safety, with backyard production systems demonstrating significantly higher contamination risks for both external

and internal egg compartments. Of note, some eggs yielded *E. coli* from both the shell and the contents; isolates were considered distinct if recovered from different sample sites within the same egg.

Table 1. *Prevalence of E. coli Contamination by Sample Type and Production System*

Sample Type	Local Eggs (n=75)	Industrial Eggs (n=75)	Total (N=150)	p-value
Eggshell	39 (52.0%)	26 (34.7%)	65 (43.3%)	0.032
Egg content	18 (24.0%)	9 (12.0%)	27 (18.0%)	0.045

All 78 isolates (comprising 65 from shells and 13 additional unique isolates from contents) were confirmed as *E. coli* through biochemical testing. As shown in Table 2, all isolates demonstrated typical biochemical characteristics, including a metallic sheen on EMB agar, positive indole and methyl red tests, negative Voges-Proskauer and citrate utilization tests, and negative oxidase tests with positive catalase reactions, confirming the accuracy of the isolation procedure.

Table 2. *Biochemical Characteristics of Confirmed E. coli Isolates (n=78)*

Test	Expected Result	Observed Result	Reference
EMB Agar	Metallic sheen	Metallic sheen	Malkawi et al., 2017
Indole Test	Positive	Positive	Cappuccino & Welsh, 2017
Methyl Red Test	Positive	Positive	Cappuccino & Welsh, 2017
Voges-Proskauer Test	Negative	Negative	Cappuccino & Welsh, 2017
Citrate Utilization	Negative	Negative	Cappuccino & Welsh, 2017
Oxidase Test	Negative	Negative	Pires et al., 2018
Catalase Test	Positive	Positive	Prescott et al., 2021

All 78 confirmed *E. coli* isolates were successfully tested against a panel of seven antimicrobial agents using the disk diffusion method with CLSI breakpoints. Resistance rates varied considerably across antibiotics, as detailed in Table 3. High resistance rates were observed for tetracycline (76.9%) and ampicillin (69.2%). Moderate resistance was detected for chloramphenicol (38.5%) and the third-generation cephalosporin cefotaxime (29.5%). Notably, ciprofloxacin and gentamicin—both critically important antimicrobials for human medicine—retained the highest efficacy, with resistance rates of 11.5% and 15.4%, respectively.

Table 3. *Antimicrobial Resistance Profiles of E. coli Isolates (n=78)*

Antimicrobial Agent	Disk Potency (µg)	CLSI Breakpoint (mm)	Resistant Isolates n (%)	95% CI
Tetracycline	30	≤11 (R)	60 (76.9)	[66.0, 85.8]
Ampicillin	10	≤13 (R)	54 (69.2)	[57.8, 79.2]
Chloramphenicol	30	≤12 (R)	30 (38.5)	[27.7, 50.0]
Cefotaxime	30	≤22 (R)	23 (29.5)	[19.7, 40.8]
Gentamicin	10	≤12 (R)	12 (15.4)	[8.2, 25.3]
Ciprofloxacin	5	≤15 (R)	9 (11.5)	[5.4, 20.8]

Note: CLSI = Clinical and Laboratory Standards Institute; R = Resistant; CI = Confidence Interval

When resistance profiles were compared across production systems, isolates from local eggs consistently showed higher resistance rates to all antibiotics tested (Table 4). For example, resistance to ampicillin was observed in 78.9% of local egg isolates compared to 60.0% of industrial egg isolates, while resistance to cefotaxime was nearly double in local isolates (39.5% vs. 20.0%). Although these differences did not reach statistical significance for individual antibiotics—likely due to sample size limitations—the consistent pattern of higher resistance in local egg isolates was evident across all seven antimicrobial agents, emphasizing the impact of production practices and antimicrobial use patterns.

Table 4. *Antimicrobial Resistance of E. coli by Egg Production System*

Antimicrobial Agent	Resistance in Local Eggs (n=38)	Resistance in Industrial Eggs (n=40)	p-value
Tetracycline	32 (84.2%)	28 (70.0%)	0.137
Ampicillin	30 (78.9%)	24 (60.0%)	0.071
Chloramphenicol	18 (47.4%)	12 (30.0%)	0.114
Cefotaxime	15 (39.5%)	8 (20.0%)	0.059
Gentamicin	7 (18.4%)	5 (12.5%)	0.466
Ciprofloxacin	5 (13.2%)	4 (10.0%)	0.735

Multidrug resistance, defined as resistance to three or more antimicrobial classes, was prevalent among the *E. coli* isolates. As shown in Table 5, 45 of 78 isolates (57.7%) were MDR. Strikingly, MDR was significantly more common in isolates from local eggs compared to those from industrial eggs. Among the 38 isolates from local eggs, 26 (68.4%) were multidrug-resistant, whereas only 19 of the 40 isolates from industrial eggs (47.5%) exhibited MDR ($p = 0.047$). The most common MDR patterns observed included combined resistance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, with some isolates demonstrating resistance to five or more antimicrobial classes. These findings demonstrate that not only are local eggs more frequently contaminated with *E. coli*, but the contaminating strains are also significantly more likely to exhibit multidrug resistance, representing a compounded public health risk.

Table 5. *Multidrug Resistance Patterns by Production System*

MDR Category	Local Eggs (n=38)	Industrial Eggs (n=40)	Total (N=78)	p-value
MDR (≥3 classes)	26 (68.4%)	19 (47.5%)	45 (57.7%)	0.047
Non-MDR (<3 classes)	12 (31.6%)	21 (52.5%)	33 (42.3%)	

DISCUSSION

This study provides the first comprehensive data on Escherichia coli contamination and antimicrobial resistance in table eggs sold in Kabul, Afghanistan, revealing concerning levels of both microbial contamination and drug-resistant bacteria in a critical food source. The findings have significant implications for food safety, public health, and antimicrobial stewardship in a country with limited regulatory infrastructure and surveillance capacity.

The overall contamination rates of 43.3% on eggshells and 18.0% in egg contents are alarmingly high and exceed those reported in many studies from countries with established

food safety systems. These findings align with global reports highlighting eggs as potential vehicles for foodborne pathogens, especially when proper hygiene and handling practices are lacking (Dognon et al., 2026; Dushayeva, 2025; Rafiq et al., 2024). The higher contamination on shells compared to contents is expected, as the shell is the first line of defense and is directly exposed to environmental contaminants, including fecal matter, during laying and collection.

The significantly higher contamination in local eggs compared to industrial eggs (52.0% vs. 34.7% on shells; 24.0% vs. 12.0% in contents) is consistent with previous studies conducted in similar low-resource settings. Research from Bangladesh, India, and Nigeria has similarly demonstrated that local or backyard eggs are more prone to contamination due to poor sanitary conditions and limited regulatory oversight (Rafiq et al., 2024; Taskeen et al., 2025; Oladipo et al., 2026). Several factors likely contribute to this disparity, beginning with the production environment. Local birds are typically raised in backyard systems with uncontrolled access to environments contaminated with feces, soil, and other potential sources of E. coli. Hens often scavenge for food and may be housed in proximity to other domestic animals, facilitating pathogen transmission (Wong et al., 2017; Zahir et al., 2024).

Egg handling practices further exacerbate contamination risks in local production systems. Eggs are typically collected manually, often infrequently, and may remain in nesting areas for extended periods, allowing bacterial penetration through the porous shell. Unlike industrial operations, local eggs are rarely washed or sanitized before sale and are transported and stored without refrigeration (Taskeen et al., 2025). This absence of cold chain maintenance is particularly concerning, as industrial eggs benefit from temperature control from production through retail, while local eggs are typically held at ambient temperatures. Such temperature abuse allows any contaminating bacteria to proliferate, increasing both the likelihood of detection and the potential infectious dose for consumers (von Kiparski et al., 2025).

These findings underscore that interventions targeting the informal production sector could substantially reduce contamination risks. Practical measures include educating farmers on basic hygiene, providing clean nesting materials, and training them on the importance of frequent egg collection and cool storage. Such low-cost interventions have the potential to improve food safety in resource-limited settings significantly.

The high resistance rates observed, particularly to tetracycline (76.9%) and ampicillin (69.2%), are deeply concerning and consistent with findings from other LMICs where antibiotic use in poultry is poorly regulated (Dushayeva, 2025; Rafiq et al., 2024; Taskeen et al., 2025). These three antibiotics are among the most commonly available and inexpensive antimicrobials in Afghanistan, and their widespread use in both human medicine and animal agriculture has created strong selective pressure for resistant E. coli strains.

The moderate resistance to chloramphenicol (38.5%) is noteworthy, as this antibiotic has been restricted or banned for use in food-producing animals in many countries due to its

potential to cause aplastic anemia in humans. Its continued availability and use in Afghanistan—whether in human medicine or agriculture—is suggested by this resistance level and warrants regulatory attention (Oladipo et al., 2026).

Of particular concern is the resistance to cefotaxime (29.5%), a third-generation cephalosporin classified by WHO as a "critically important antimicrobial" for human medicine (WHO, 2017). Resistance to this class of drugs, often mediated by extended-spectrum beta-lactamases (ESBLs), severely limits treatment options for serious infections caused by E. coli, including sepsis and complicated urinary tract infections. The detection of cefotaxime-resistant E. coli in eggs destined for human consumption suggests that ESBL-producing strains have already entered the food chain in Afghanistan, mirroring trends observed in other Asian countries (Taskeen et al., 2025; Yue et al., 2024).

The relatively lower resistance rates to ciprofloxacin (11.5%) and gentamicin (15.4%) provide some reassurance, though these levels are not negligible. Ciprofloxacin is a frontline antibiotic for many bacterial infections, and emerging resistance, even at current levels, warrants close surveillance and precautionary use in both human and veterinary sectors (Clinical and Laboratory Standards Institute, 2023). The lower resistance may reflect the higher cost and more restricted availability of these drugs in Afghanistan. However, trends from other countries suggest that resistance to these "last-line" drugs can rise rapidly if stewardship measures are not implemented (Dognon et al., 2026).

The finding that 57.7% of isolates were multidrug-resistant, with significantly higher rates in local eggs (68.4% vs. 47.5%), represents a major public health concern. MDR organisms pose a dual threat: they can cause infections that are difficult or impossible to treat with conventional antibiotics, and they serve as reservoirs of resistance genes that can be transferred horizontally to other bacteria via mobile genetic elements (Dushayeva, 2025; Tola et al., 2026).

The higher MDR rates in isolates from local eggs likely reflect the cumulative effects of several factors characteristic of informal production systems: uncontrolled access to antibiotics, inappropriate dosing, lack of veterinary oversight, and nonobservance of withdrawal periods. In such settings, antibiotics are often used indiscriminately—administered without diagnosis, in subtherapeutic doses, and for growth promotion—creating ideal conditions for the selection and amplification of MDR strains (Rafiq et al., 2024; von Kiparski et al., 2025).

The public health implications extend beyond the immediate risk of foodborne infection. E. coli from food animals can colonize the human gut, where it may persist and serve as a continuous source of resistance genes. These genes can be transferred to commensal flora and, under selective pressure from antibiotic use in humans, to pathogenic bacteria, further complicating treatment of both community-acquired and healthcare-associated infections (Oladipo et al., 2026; Pooja Sajish et al., 2025).

Strengths and Limitations

This study has several strengths, including its representative sampling across diverse retail sources, differentiation between production systems, separate analysis of eggshell and content contamination, and application of standardized CLSI methodologies for susceptibility testing. The use of a broad antibiotic panel, including critically important antimicrobials, provides valuable baseline data for future surveillance.

However, several limitations must be acknowledged. First, the study sample, while representative of multiple retail sources, was restricted to Kabul city and may not reflect conditions in other provinces of Afghanistan. Seasonal variations in microbial prevalence were not assessed because sampling was limited to 3 months. Second, while phenotypic antimicrobial susceptibility testing is standard, it cannot identify the underlying genetic mechanisms of resistance (e.g., specific resistance genes and plasmid profiles), which would provide valuable information for understanding the epidemiology and transmission dynamics of resistant strains. Third, the absence of genotypic characterization (e.g., multilocus sequence typing, whole-genome sequencing) precludes identification of specific pathotypes or phylogenetic groups that may have enhanced virulence potential. Fourth, the relatively small number of isolates per production system limited statistical power to detect differences in resistance rates for individual antibiotics, despite the consistent pattern observed. Fifth, this study did not investigate antibiotic residues in eggs or antibiotic usage patterns on farms, which would help establish clearer links between agricultural practices and observed resistance.

Recommendations

The findings of this study underscore the urgent need for coordinated, multisectoral interventions within a One Health framework that integrates animal health, food safety, and public health systems in Afghanistan.

At the poultry production level, particularly in backyard and smallholder systems, implementing cost-effective biosecurity measures is essential to reduce egg contamination. Maintaining clean nesting environments, ensuring frequent egg collection, and minimizing contact between poultry and other livestock species should be standardized practices. Capacity-building initiatives are needed to improve farmers' awareness of hygienic egg handling and responsible antimicrobial use. Enforcement of antibiotic withdrawal periods in laying hens must be strengthened to reduce antimicrobial residues and selection pressure. In parallel, antimicrobial stewardship should be promoted through preventive strategies, including improved husbandry, vaccination, and evidence-based alternatives such as probiotics.

At the national level, the development and strict enforcement of regulatory frameworks governing antimicrobial use in food-producing animals are critical. Over-the-counter access to critically important antimicrobials should be restricted, and veterinary oversight reinforced. Establishing a national antimicrobial resistance surveillance system targeting foodborne pathogens is strongly recommended, with eggs designated as a sentinel commodity due to their widespread consumption and distribution. Standardized cold-chain

requirements for egg transport and storage should also be implemented and monitored to limit post-production bacterial proliferation.

Within the public health and clinical sectors, enhanced awareness of foodborne transmission of antimicrobial-resistant pathogens is necessary to guide empirical therapy and infection control strategies. Strengthening laboratory capacity for reliable isolation, identification, and antimicrobial susceptibility testing is essential. Integration of food safety surveillance data with national antimicrobial resistance monitoring systems would enable the development of evidence-based treatment guidelines and more effective public health interventions.

Future research should focus on molecular characterization of resistant *Escherichia coli* isolates to identify resistance determinants and transmission pathways. Longitudinal studies assessing seasonal trends and antibiotic usage patterns in poultry production are warranted to clarify drivers of resistance emergence. Expanded surveillance to include other major foodborne pathogens and quantitative risk assessment studies will further support evidence-based policymaking and national action plans against antimicrobial resistance.

CONCLUSION

This study demonstrates that eggs sold in Kabul, Afghanistan, are frequently contaminated with Escherichia coli, and that a substantial proportion of these isolates exhibit resistance to multiple clinically important antibiotics, including those classified as critically important for human medicine. The higher contamination and resistance rates in eggs from local production systems highlight the vulnerabilities associated with informal poultry keeping and the urgent need for targeted interventions. These findings provide essential baseline data for developing evidence-based food safety policies, antimicrobial stewardship programs, and public health strategies in Afghanistan. Addressing the dual challenges of microbial contamination and antimicrobial resistance in the food supply will require a coordinated "One Health" approach engaging the agricultural, veterinary, environmental, and human health sectors.

AUTHOR'S CONTRIBUTIONS

Ezatullah Jaheed and Said Arif Ahmadi jointly designed the study and supervised its overall implementation. Said Arif Ahmadi was responsible for the collection of egg samples from various retail sources. Ezatullah Jaheed conducted the laboratory processing of egg samples and performed the biochemical identification tests. Said Arif Ahmadi carried out the antimicrobial susceptibility testing of the isolates. Ezatullah Jaheed performed the data analysis and interpretation of the results. Both Ezatullah Jaheed and Said Arif Ahmadi contributed to the writing and revision of the manuscript. All authors read and approved the final version of the manuscript

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding this study.

DATA AVAILABILITY STATEMENT

The data can be accessed upon request from the corresponding author, with the approval of the appropriate ethics committee.

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