Prevalence and Antimicrobial Resistance Pattern of *Salmonella* Isolated from Table Eggs in Chitwan District, Nepal

Abstract

This study aimed to investigate the prevalence and antimicrobial resistance patterns of *Salmonella* in table eggs in the Chitwan district. Between September and December 2022, a total of 146 table eggs were collected from different retail shops and layer farms within the district—40 eggs from layer farms and 106 from retail shops. All 146 eggs were tested for *Salmonella* using standard culture-based methods.

The overall prevalence of *Salmonella* in table eggs was found to be 6.16%. Among eggs obtained from retail shops, *Salmonella* was isolated from 2.73% of eggshells (shell only), 1.36% of egg contents (content only), and 1.36% of samples in which both the shell and the content tested positive. In contrast, eggs collected from layer farms exhibited a substantially lower prevalence, with *Salmonella* detected in 0.68% of eggshells only. No *Salmonella* was recovered from egg contents or from both components in the layer farm samples.

The resistance of *Salmonella* to Ceftriaxone, Tetracycline, Chloramphenicol, Enrofloxacin, and Ciprofloxacin was 9.1%, 27.3%, 18.2%, 100%, and 36.3%, respectively. Additionally, 18.18% of the *Salmonella* isolates were resistant to three or more antibiotic groups, indicating multidrug resistance. Notably, all multidrug-resistant *Salmonella* were isolated from eggshells.

The results of this study indicate a higher prevalence of *Salmonella* in eggs from retail markets, suggesting a greater risk to consumers. Reducing the *Salmonella* contamination rate in retail eggs requires effective interventions at both the farm and packing station levels. Moreover, eggs should be thoroughly cooked before consumption to minimize health risks.

Keywords: *AMR, egg contamination, MDR, Public health, Zoonosis, Salmonella, Chitwan, Retail Shop*

1. Introduction

1.1 Background

Non-typhoidal *Salmonella* is one of the most commonly reported enteric pathogens worldwide (Tauxe & Pavia, 1998). The disease is estimated to cause approximately 153 million cases of gastroenteritis and 57,000 deaths globally each year (Jessica et al., 2022). In 2018, *Salmonella* was implicated in 30.7% of reported foodborne outbreaks, making it the second most commonly reported zoonotic disease leading to hospitalization after campylobacteriosis, and the second leading cause of death after listeriosis, due to the consumption of contaminated food in Europe (EFSA and ECDC, 2019). Several foods have been linked to outbreaks of salmonellosis (Ferrari et al., 2019).

Poor implementation of biosecurity in poultry farm also increases the risk of zoonotic pathogens like Salmonella entering the food chain (Dhakal et al., 2025). Consumption of undercooked or raw eggs has been identified as a significant risk factor for salmonellosis, contributing to 47.2% of total *Salmonella* infections (Ferrari et al., 2019). In most developed countries, the prevalence of *Salmonella* in commercial table eggs is minimal (Harsha, 2011). Poultry birds are frequently infected with *Salmonella*, making them a major source of human infection (Vandeplas et al., 2010). The use of antimicrobial agents to treat salmonellosis in poultry has led to the emergence of *Salmonella* spp. with increased resistance to these agents (Phagoo & Neetoo, 2015). Furthermore, the routine use of antibiotics for growth promotion and prophylaxis in layer hens has contributed to the development of antibiotic-resistant bacteria (Mudenda et al., 2022).

1.2 Problem Statement

Egg-borne salmonellosis is a significant global public health issue (Rahman et al., 2019). The increase in antimicrobial resistance of Salmonella has become a worldwide problem in recent decades (Su et al., 2004). Due to the decreased effectiveness of antimicrobial treatments, antibiotic-resistant bacteria isolated from foodborne diseases like Salmonellosis are a public health problem (Sin et al., 2020). The common practices of using antibiotics for the growth and prophylaxis of layers have contributed to the development of antibiotic-resistant bacteria (Mudenda et al., 2022).

This study investigates the presence of Salmonella in eggshells and egg contents (albumin and yolk), evaluates the contamination of table eggs collected from layer farms and retail markets, examines the antibiotic resistance patterns of Salmonella isolates against commonly used antibiotics, and analyzes the multidrug resistance profiles of the isolated strains to provide insights into the public health risks associated with egg-borne salmonellosis.

2. Methodology

2.1 Study Area

The study was conducted in Chitwan District, Nepal, from September to December 2022. Samples were collected from retail egg shops and layer farms within the district. The collected samples were then transported to the laboratory of the National Avian Disease Investigation Laboratory, Chitwan.

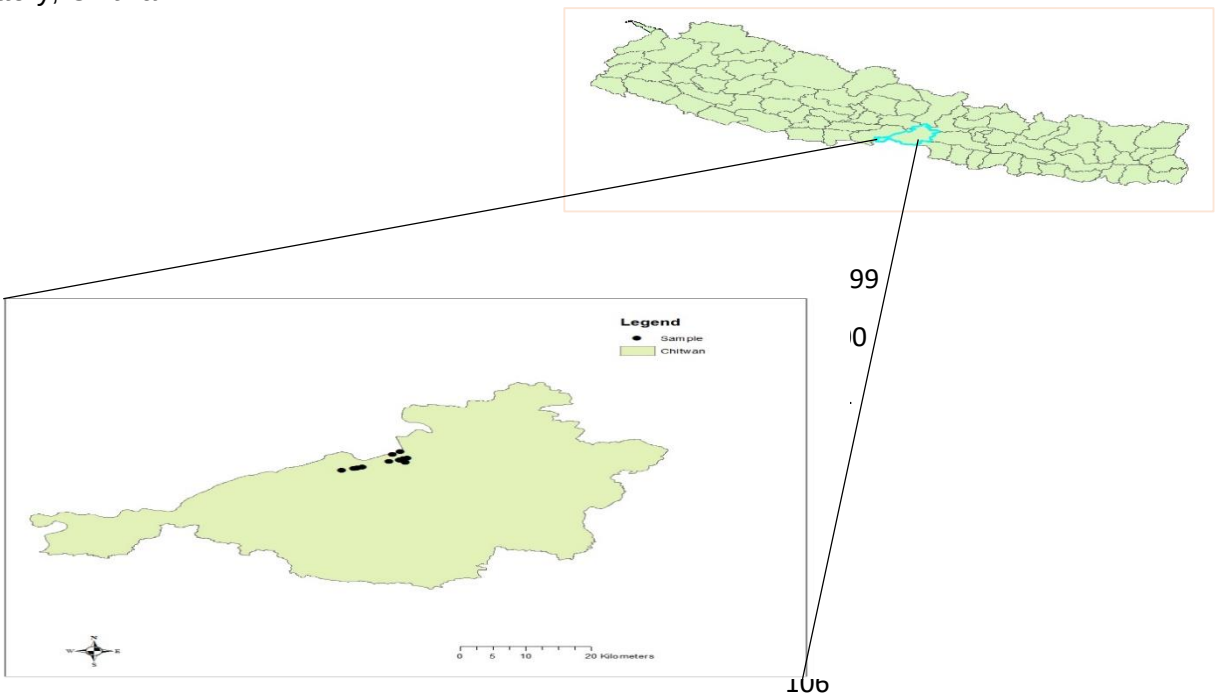

Fig. 1. Map of Nepal showing the Study area

2.2 Study Population and Sample Size

Purposive sampling was conducted in retail shops and layer farms of the Chitwan district.

Population Size: Unknown

To determine the sample size for a study with an unknown population size, the following formula was used:

$$n = \frac{Z^2 \,.\, P \,.\, (1 - P)}{e^2}$$

Where:

n = Required sample size
Z = Z-value (standard normal deviate) corresponding to the desired confidence level
P = Expected prevalence of the pathogen
e = Margin of error (desired level of precision)

Based on the findings of Sharma et al. (2021), the expected prevalence of the pathogen in poultry feces is 10.6%.

Given:
Z = 1.96 (corresponding to a 95% confidence level)
P = 0.106

e = 0.05

$$n = \frac{1.96^2 \,.\, 0.106 \,.\, (1 - 0.106)}{0.05^2}$$

Thus, the minimum required sample size is 146.

2.3 Sample Collection and Processing

2.3.1 Collection of Sample:

A total of 146 table eggs were collected, of which 40 were obtained directly from layer farms and 106 from retail shops. The samples were collected aseptically using sterile zipper bags, gloves, and other appropriate materials. During the collection process, precautions were taken at all stages—including sampling, transportation, and storage—to minimize the risk of cross-contamination.

2.3.2 Pre-enrichment

A non-selective medium, such as buffered peptone water, was used as a pre-enrichment medium in which most strains exhibit sufficient growth after incubation for 24 hours at 37°C. The eggs were cracked into sterile aluminum foil bowls using sterile scissors. The contents of the eggs (albumen and yolk) were mixed thoroughly. Then, 1 mL of the mixture was added to 9 mL of buffered peptone water using a micropipette. The corresponding eggshells were crushed, and 1 g of shell was mixed with 9 mL of buffered peptone water in a separate tube. All tubes were incubated at 37°C for 24 hours.

2.3.3 Enrichment

Selenite broth was used as enrichment media. 1 ml of pre-enriched sample was mixed with 5 ml of selenite broth and incubated at 37°C for 4-6 hours.

2.3.4 Isolation

Xylose-Lysine Desoxycholate (XLD) agar medium was used for the isolation of *Salmonella* species due to its high selectivity. A loopful of the enriched sample was streaked on XLD agar media and incubated at 37°C for 24 hours. After 24-hour incubation, the pink colonies with the black center were used for biochemical characterization.

Result of Gram Staining: In gram staining under the microscope, the organism revealed gram-negative, pink color; small rod-shaped appearance, arranged in single, paired, or chain form

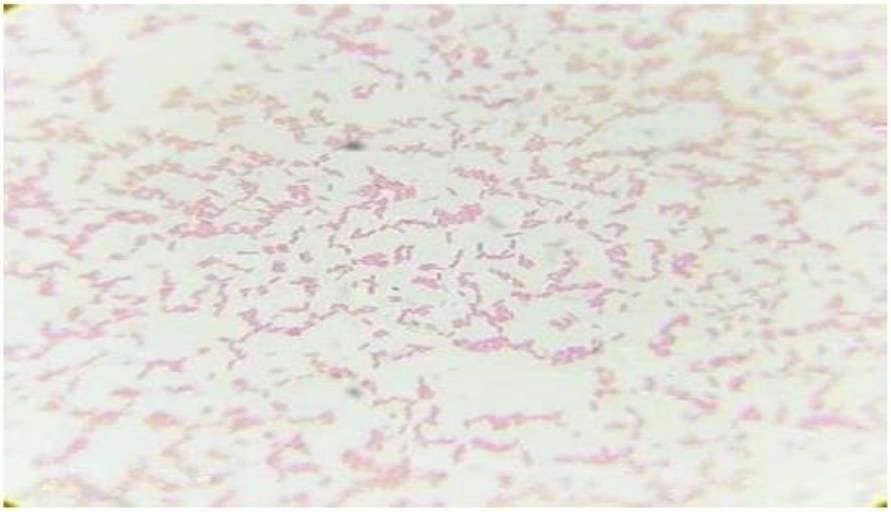

Fig. 2. Gram's stain Gram-negative medium-sized rod-shaped bacterium

Fig. 3. Enrichment of sample in selenite broth

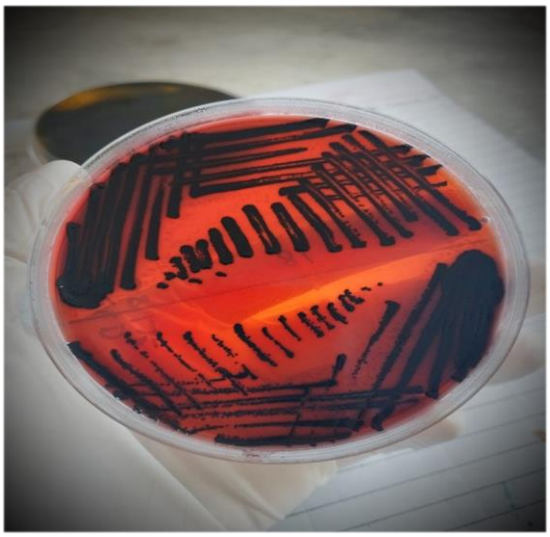

Fig. 4. Salmonella on XLD Agar

2.3.5 Biochemical tests:

To confirm the identity of suspected *Salmonella* species, a series of biochemical tests were performed. The initial screening involved Gram staining, catalase, and oxidase tests. Gram staining identified the organisms as Gram-negative. The catalase test showed bubbling when exposed to 5% hydrogen peroxide, indicating a positive reaction. The oxidase test yielded a purple coloration on the oxidase disc, also confirming a positive result.

Colonies that tested positive in these preliminary assessments underwent further biochemical testing. The Simmons Citrate test demonstrated the ability of the organism to utilize citrate, evidenced by a color change to blue. The Triple Sugar Iron (TSI) test was used to evaluate carbohydrate fermentation and hydrogen sulfide (H_2S) production, with results indicated by specific color shifts in the medium. The Sulfur Indole Motility (SIM) test was employed to examine motility, indole formation, and H_2S production, with blackening and medium turbidity signaling positive results. The Methyl Red (MR) test confirmed acid production through a red color change, while the Voges-Proskauer (VP) test, used to detect butanediol fermentation, showed no color development, indicating a negative outcome.

2.3.6 Antibiotic susceptibility test:

Each isolate was tested for its sensitivity to the following antibiotics: Chloramphenicol, Ciprofloxacin, Enrofloxacin, Tetracycline, and Ceftriaxone. Well-isolated colonies from XLD agar were inoculated into the nutrient broth and the turbidity of the suspension was adjusted to a 0.5 McFarland standard. After 15 minutes, each *Salmonella* isolates were cultured on Mueller-Hinton Agar using a sterile cotton swab. The antibiotic discs were then placed on the media. The plates were incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured in millimeters with a vernier caliper scale. Using an interpretation chart, according to the zone size of each antimicrobial reporting the organism was interpreted as 'Resistant', 'Intermediate', and 'Sensitive'.

Fig.5. Growth inhibition zone of *Salmonella* against selected antibiotic discs

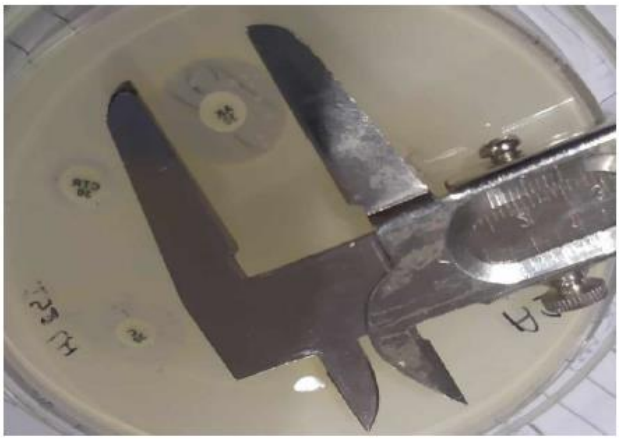

Fig.6. Determination of inhibition zone diameter using a Vernier caliper

Fig. 7. SIM media for indole, motility and sulfide test

Fig. 8. Triple Sugar Iron Test

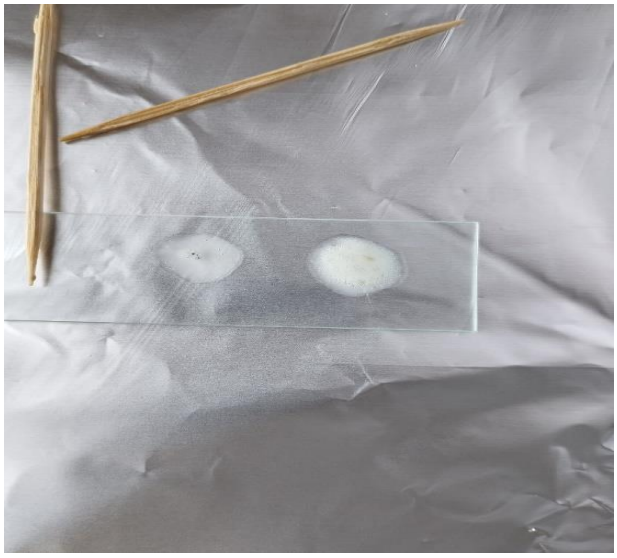

Fig.9. Urease Test

Fig.10. Catalase Test

Fig.11. Citrate Utilization Test **Fig.12 Antibiotic Sensitivity Test**

2.4 Statistical analysis:

The data were collected routinely and entered into an Excel sheet. Entered data will be analyzed using IBM SPSS Statistics version 25.

3. Result

3.1 Prevalence of *Salmonella* in table egg samples sourced from Chitwan district

Table 1: Prevalence of *Salmonella* from eggshell and contents

Collection point	Positive Samples			Negative Samples	Total No. of samples
	Shell only	Content only	Shell and Content		
Retail shops	4 (2.73%)	2 (1.36%)	2 (1.36%)	98 (67.1%)	106 (72.6%)
Layer Farms	1 (0.68%)	0 (0%)	0 (0%)	39 (26.7%)	40 (26.39%)
Total	5 (3.42%)	2 (1.36%)	2 (1.36%)	137 (93.84%)	146 (100%)
	6.16%				

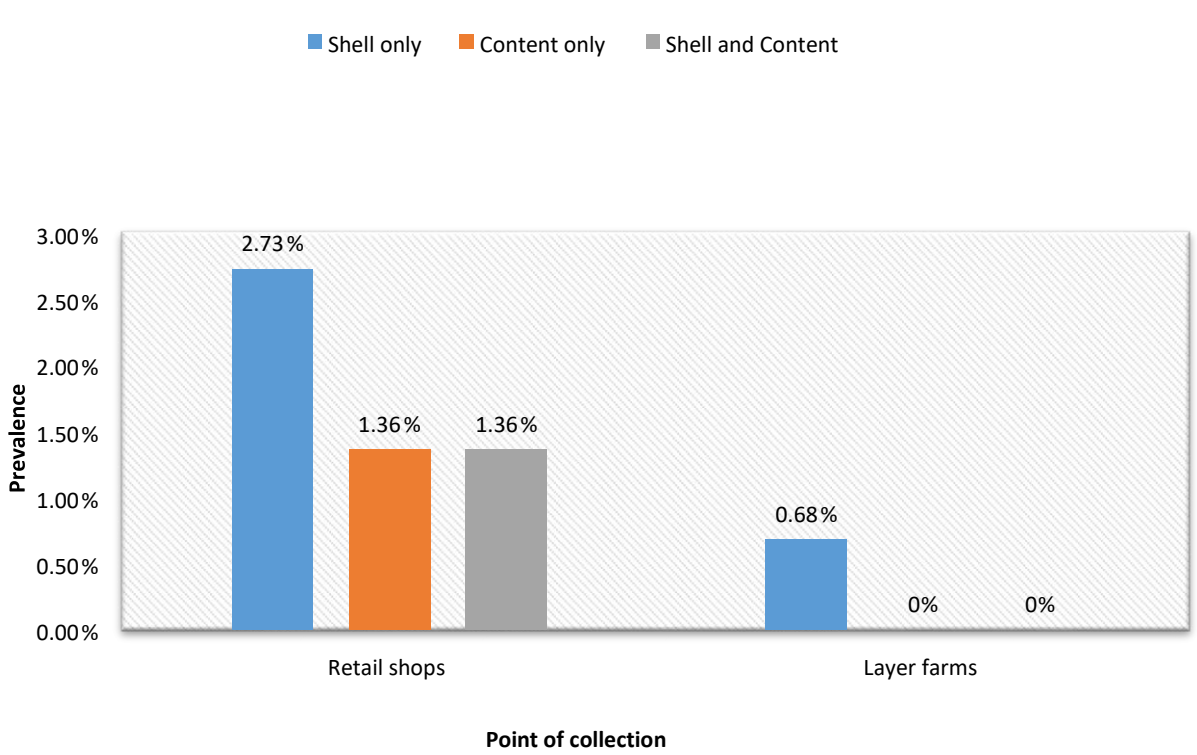

Figure 13: Prevalence of *Salmonella* in eggs

The overall prevalence of *Salmonella* in table eggs was found to be 6.16%. Among eggs obtained from retail shops, *Salmonella* was isolated from 2.73% of eggshells (shell only), 1.36% of egg contents (content only), and 1.36% of samples in which both the shell and the content tested positive. In contrast, eggs collected from layer farms exhibited a substantially lower prevalence, with *Salmonella* detected in 0.68% of eggshells only. No *Salmonella* was recovered from egg contents or from both components in the layer farm samples.

3.2 Prevalence of *Salmonella* in shell and content samples

7 (4.8%) of 146 eggshells and 4 (2.7%) of 146 egg content samples tested positive for Salmonella.

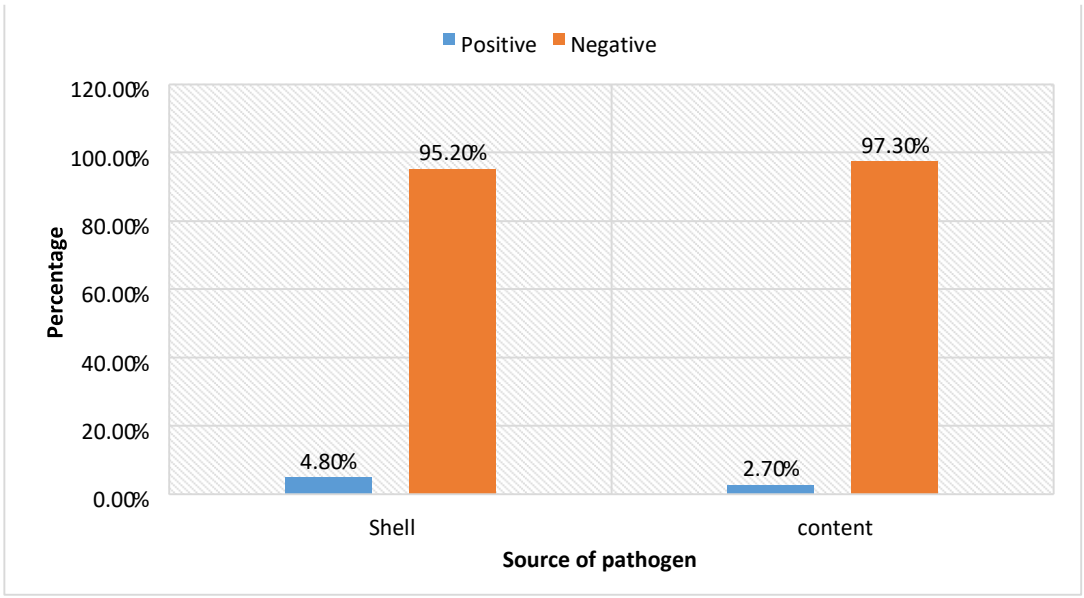

Figure 14: Frequency of Salmonella in shell and content

Table 2. Statistical association of *Salmonella* with sample type

Sample type	Number of sample (X)	Number of positive (Y)	Prevalence (Y/X)	Odds ratio (95% CI)	*p*-value	Association
Shell	146	7	4.79%	1.78 (0.45–7.04)	0.54	Statistically non-significant(*p*> 0.05)
Content	146	4	2.74%	—	—	—

The result showed that there is no significant difference in prevalence of Salmonella between eggshell and content samples examined (p > 0.05).

3.3 Prevalence of *Salmonella* in table eggs sourced from retail shops and layer farms

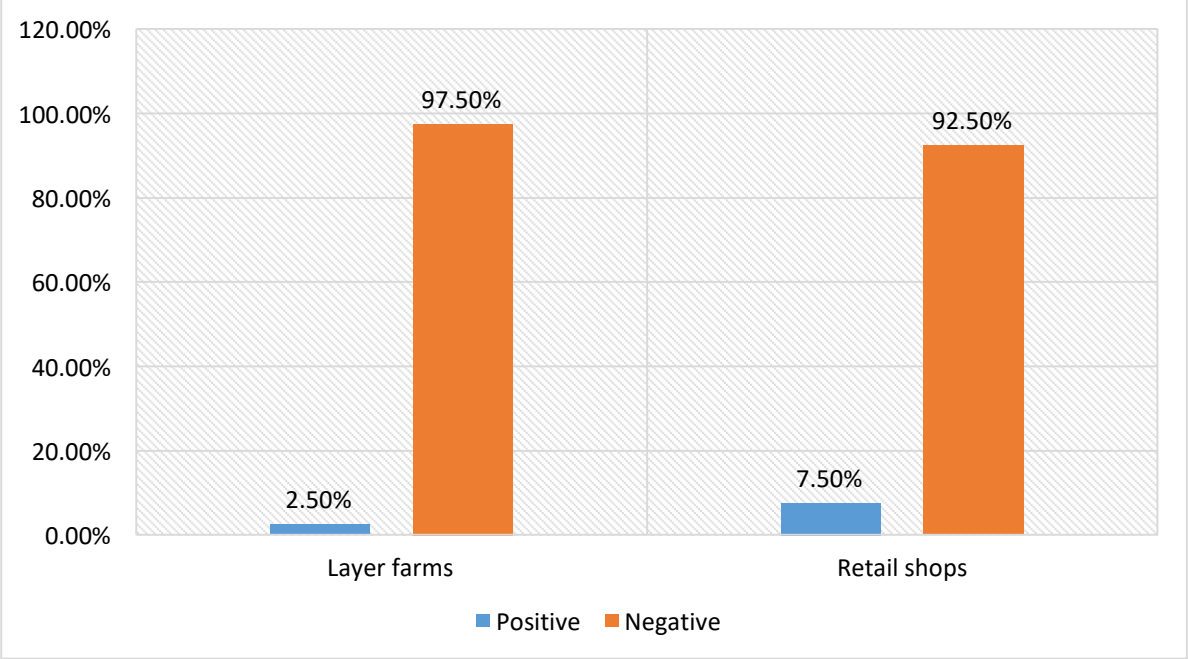

Figure 15: Prevalence of n table eggs sourced from retail shops and layer farms

Table 3. Statistical Association of *Salmonella* with Collection Point

Collection Point	*Number of samples (X)*	*Number of positive (Y)*	*Prevalence (Y/X)*	*Odds Ratio (95% CI)*	*p-value*	*Association*
Retail Shops	*106*	*8*	*7.55%*	*3.18 (0.38–26.36)*	*0.28*	*Statistically non-significant*
Layer Farms	*40*	*1*	*2.50%*	—	—	

The result shows that there is no significant difference in prevalence of Salmonella between

316 *eggs collected from retail shops and layer farms (p > 0.05).*
317

318 The prevalence of *Salmonella* in eggs collected from **layer farms** was found to be **2.50%.** The
319 prevalence of *Salmonella* in eggs collected from the **retail market** was found to be **7.50%**.

320 **3.4 Antimicrobial resistance profiles of *Salmonella* isolated from table eggs:**

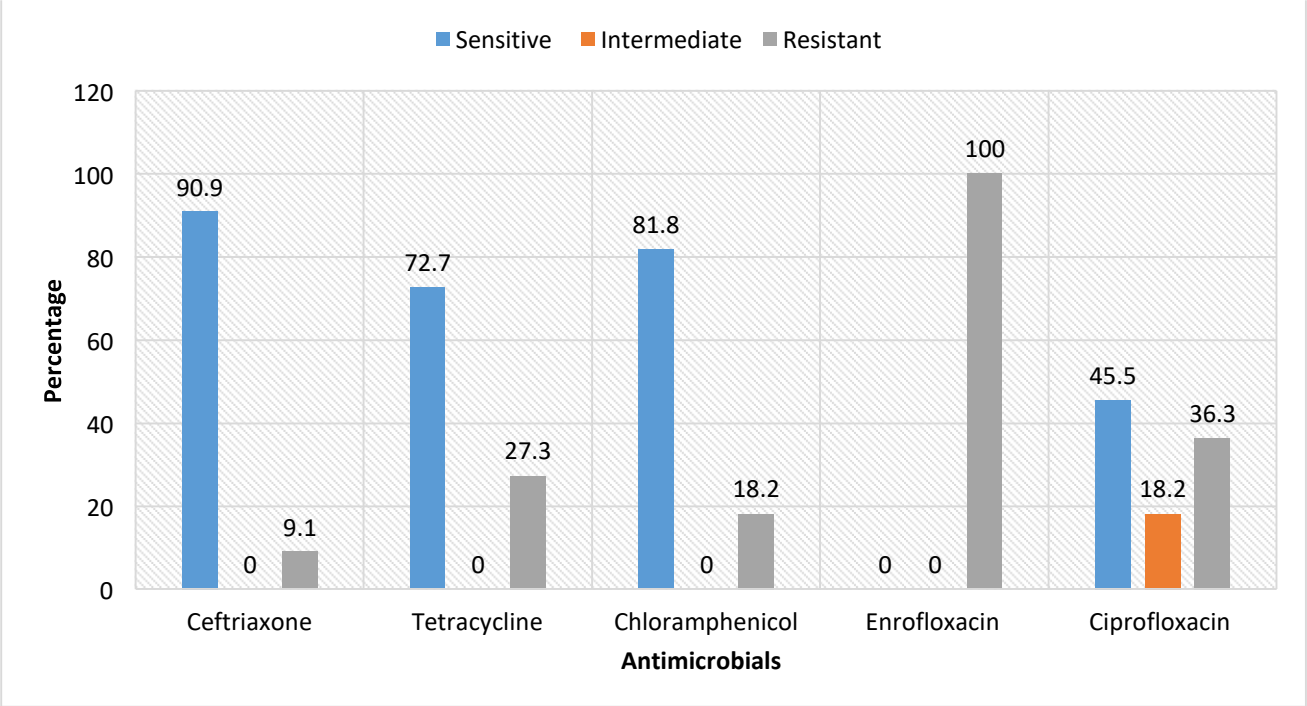

Figure 16: Antimicrobial resistance profiles of Salmonella isolated from Table eggs

321 The resistance of the *Salmonella* to Ceftriaxone, Tetracycline, Chloramphenicol, Enrofloxacin,
322 and Ciprofloxacin was found to be 9.1%, 27.3%, 18.2%, 100%, and 36.3% respectively.

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324 **3.5 Multi-drug resistance profiles of *Salmonella* isolated from table eggs**

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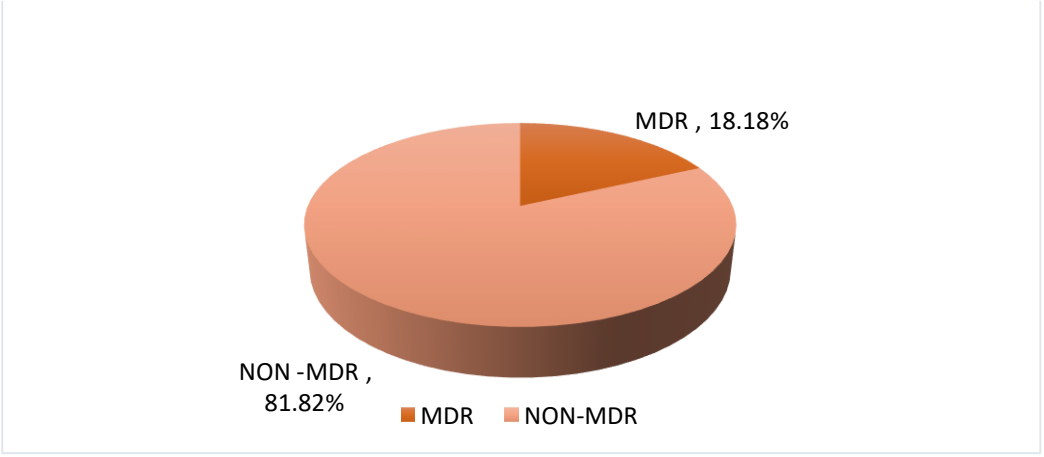

326
327 **Figure 17: Multi-drug resistance profiles of *Salmonella* isolated from table eggs**

18.18% isolated *Salmonella* were found to be resistant to ≥3 antibiotic groups. All of the MDR *Salmonella* were recovered from eggshells.

4. Discussion

The present study found an overall *Salmonella* prevalence of 6.16% in table eggs collected from Chitwan, Nepal, with a notably higher prevalence in retail shop samples (7.55%) than in farm-collected eggs (2.5%). This difference suggests that contamination is more likely to occur during post-farm handling, such as transportation, storage, and display at markets. Similar findings were reported by El Ftouhy et al. (2022), who observed significantly higher bacterial contamination in eggs from informal markets than from formal sources in Morocco. This may be due to poor hygiene practices, lack of refrigeration, and frequent human contact with eggs during retail handling.

The lower prevalence in farm eggs observed in our study may be due to better on-site hygiene and biosecurity. Dhakal et al. (2025) emphasized that poultry farms with improved biosecurity in Chitwan had a reduced risk of *Salmonella* contamination. Similarly, Shah et al. (2021) found that the prevalence of *Salmonella* was lower in eggs collected directly from farms compared to retail outlets in Peshawar, Pakistan.

Shell contamination (4.79%) was more common than contamination of egg contents (2.74%), which aligns with previous research. Gantois et al. (2009) reported that eggshells are more likely to be contaminated during or after laying, especially when they come into contact with feces, dirty nesting materials, or human hands. Contamination of the contents usually occurs when bacteria penetrate the shell through cracks or pores, which is less common. Bruce and Drysdale (1994) and Messens et al. (2007) both observed that environmental conditions, like high humidity and improper washing, can facilitate the movement of *Salmonella* through the eggshell barrier.

Although our statistical analysis showed no significant difference in contamination between shell and content samples ($p > 0.05$), the practical risk is considerable. Contaminated shells can lead to cross-contamination during food preparation, especially when raw eggs are handled or undercooked. This is consistent with the findings of Howard et al. (2012), who stated that shell contamination remains a key route for *Salmonella* entry into households.

Antimicrobial resistance (AMR) patterns showed that all *Salmonella* isolates were resistant to Enrofloxacin (100%), and many were resistant to Ciprofloxacin (36.3%), Tetracycline (27.3%), and Chloramphenicol (18.2%). This trend is alarming but not surprising. Phagoo and Neetoo (2015) found that the overuse of antibiotics like Enrofloxacin in poultry farms contributed to high resistance rates in *Salmonella* isolates. Similar resistance to fluoroquinolones has been documented by Sin et al. (2020) in Korea, and by Haque et al. (2021) in Bangladesh.

Additionally, 18.18% of the *Salmonella* isolates in our study were multidrug-resistant (MDR), meaning they were resistant to three or more antibiotic classes. All MDR isolates were found on eggshells. This suggests that MDR strains are more likely to be acquired from external environmental sources, such as contaminated surfaces, farm litter, or human handling, rather than vertical transmission through the egg contents. Rahman et al. (2019) similarly reported that MDR *Salmonella* was more frequently recovered from eggshells than from the internal contents.

Our prevalence rate (6.16%) falls between findings from different regions. It is lower than the 11.5% reported by Shah et al. (2021) in Pakistan but higher than the 2.5% found by Harsha (2011) in India. These differences may be due to variations in biosecurity levels, antibiotic use, temperature and humidity during storage, and national food safety regulations. In countries

with strict egg handling protocols and routine refrigeration, such as the United States or the EU, contamination rates tend to be much lower (Howard et al., 2012).

In summary, the results highlight that eggs from retail outlets are more likely to be contaminated with *Salmonella*, particularly on the shell, and that some of these isolates show resistance to multiple antibiotics. This suggests an urgent need for better hygiene practices at all levels of egg production and distribution, as well as careful monitoring and regulation of antibiotic use in poultry farms to prevent the spread of resistant *Salmonella* strains.

5. Recommendation

To further support the findings of this study, future research should explore the epidemiology of *Salmonella* in layer farm environments and its potential transmission to humans.

6. Beneficiaries:

Primary Beneficiaries: Layer Farmers and Consumers

Secondary Beneficiaries: Researchers

7. Conclusion

The higher prevalence of *Salmonella* in eggs from retail markets suggests an increased risk to consumer health. Contamination on the eggshell surface can lead to the spread of the bacteria, either directly—through contact with hands, kitchen tools, or surfaces—or indirectly, when the bacteria enter food as the eggs are broken. To reduce *Salmonella* contamination in retail eggs, effective control measures should be applied at egg packing and processing stations. Retailers should also follow safety practices, and consumers must be encouraged to cook eggs thoroughly and prevent cross-contamination during food preparation.

8. DISCLAIMER (ARTIFICIAL INTELLIGENCE):

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS DISCLAIMER:
Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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