Food Science of Animal Products

Research Article

Dietary florfenicol residues from eggs drive multidrug resistance in *Salmonella* and enrich intestinal antibiotic resistance genes

Yan Gong, Wenduo Han, Jie Bai, Xuebo Liu, Xiang Duan ✉

College of Food Science and Engineering, Northwest A&F University, Yangling 712100, China

✉Address correspondence to Xiang Duan, duanxiang402@163.com

Received: March 26, 2026; Revised: May 20, 2026; Accepted: May 30, 2026

Abstract: This study comprehensively investigates the molecular and ecological mechanisms by which florfenicol (FFC) residues drive multidrug resistance (MDR) in *Salmonella* and the intestinal microbiota. By integrating long-term *in vitro* induction with an *in vivo* mouse model of dietary exposure, we evaluated the risk of resistance development of FFC residues. *In vitro* results demonstrated that continuous FFC pressure rapidly evolved susceptible *Salmonella* into highly resistant strains, with minimum inhibitory concentrations escalating from 8 to ≥ 64 mg/L. Crucially, this process triggered broad cross-resistance to non-targeted antibiotics, including quinolones and β-lactams. Real-time quantitative polymerase chain reaction analysis confirmed this phenotype was primarily driven by the significant overexpression of transmembrane efflux pump genes (*fexA*, *tetA*) and the ribosomal protection gene *optrA*. At the microecological level, metagenomic sequencing revealed that FFC exposure induced a dramatic expansion of the intestinal resistome. Strategies for resistance converged on the comprehensive upregulation of ATP-binding cassette, resistance-nodulation-division, and major facilitator superfamily efflux pump families, alongside target modification mechanisms. Furthermore, FFC exposure significantly disrupted intestinal homeostasis, driving the microbial community toward a dysbiotic state dominated by opportunistic pathogens harboring abundant resistance genes, specifically Proteobacteria and Enterobacteriaceae. In conclusion, FFC acts as a potent stressor that not only induces MDR at the single-bacterium level through efficient efflux and target protection but also synergistically enriches broad-spectrum resistance genes and reshapes the host microbiome structural composition. These findings highlight the critical ecological risks of FFC residues in the food chain and underscore the need for strict residue control.

Keywords: florfenicol; *Salmonella*; metagenomics; antibiotic resistance gene

Citation: Y. Gong, W. D. Han, J. Bai, et al., Dietary florfenicol residues from eggs drive multidrug resistance in *Salmonella* and enrich intestinal antibiotic resistance genes, Food Sci. Anim. Prod. 4 (2026) 9240179. https://doi.org/10.26599/FSAP.2026.9240179.

1 Introduction

Florfenicol (FFC) is a broad-spectrum amphenicol antibacterial drug specifically for animals. It features rapid absorption, high safety, significant efficacy, and potent antibacterial efficacy against bacterial diseases and mycoplasma infections in livestock, poultry, and aquatic animals. However, FFC distributes widely *in vivo* with a long half-life. This may lead to residues in animal tissues. Consequently, this triggers bacterial resistance[1]. In recent years, unreasonable use of FFC occurs in some breeding enterprises in China. This is driven by economic interests or a lack of veterinary knowledge. Therefore, FFC residues are frequently detected in animal-derived foods[2].

Food chain transmission serves as a critical pathway for the dissemination of antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) under the One Health framework[3]. Many studies show abundant ARGs/ARBs in animal-derived foods. Recent studies reveal those foods (like meat, vegetables, and fruits) host ARGs and ARBs. These foods can spread ARGs and ARBs to humans. This occurs through direct or indirect contact with contaminated food. For example, recent global surveys have frequently detected methicillin-resistant *Staphylococcus aureus* and various ARGs in retail meats. Studies highlight that poultry and pork products often exhibit significantly high detection rates of these multidrug-resistant strains, posing a direct and critical threat to public health through the food chain[4]. Final products (3%) had lower rates than processed meats (4.2%). Even processed ready-to-eat foods still carry ARGs. These genes can transfer to human intestinal bacteria. This happens through horizontal gene transfer. Mobile genetic elements (MGEs) like plasmids, transposons, and integrons mediate this process[5]. Antibiotics are widely used today. Consequently, ARBs are isolated from various animal species. Multiple ARGs have also been identified. Antibiotic resistance is a specific ability of bacteria. They resist antibiotics through gene mutations or acquired resistance genes. Currently, several ARGs are associated with FFC. These include major facilitator superfamily (MFS) efflux pumps (e.g., *floR*, *fexA*, *fexB*). They also include the rRNA methyltransferase family (e.g., *cfr*)[6] and the ATP-binding cassette (ABC) family (e.g., *optrA*)[7]. The *estDL136* gene, which encodes chloramphenicol acetate esterase, is also involved. Studies confirm that the *fexA* gene mediates resistance to chloramphenicol and FFC. The *poxtA* gene generates resistance to phenicols, oxazolidinones, and tetracyclines. Environmental studies on the *fexA* gene show its specific location[8]. It is located in a novel transposon, Tn558, on plasmids. It easily spreads among different bacterial species via transposons and plasmids. Reports indicate that FFC treatment increases homologous resistance. It also affects resistance to at least one other class of antibiotics. Bacteria develop

FFC resistance mainly through two mechanisms: reduced membrane permeability and mutations in the 50S ribosomal subunit[9]. Decreased membrane permeability reduces FFC uptake. It also promotes FFC efflux. This minimizes FFC-induced cellular damage[10]. Mutations in the 50S ribosomal subunit reduce FFC affinity for ribosomes, thereby reducing cellular damage. For example, the *floR* gene, encoding the FloR protein, belongs to the MFS family[11]. FloR is a membrane protein with 12 transmembrane hydrophobic regions. FloR specifically recognizes and transports FFC. It uses hydrophobic amino acids in its transmembrane regions. Hydrophobic residues at positions 160 and 228 of FloR play a key role in promoting the specific efflux of FFC and chloramphenicol in *Escherichia coli*. Genes can upregulate components of the resistance-nodulation-division (RND) efflux system[12], such as *AcrB* and *AcrA*, thereby enhancing bacterial resistance to FFC. This mechanism also resists other antibiotics like ciprofloxacin. The *cfr*-mediated resistance mechanism alters methylation levels of the 23S rRNA[13], reducing FFC affinity for the peptidyl transferase center. Microbes treated with FFC show many mutations related to transmembrane transport and DNA recombination. Drug/metabolite transporter superfamily permeases increase significantly. Single nucleotide mutations cover key genes in homologous recombination, including single-stranded DNA binding proteins, DNA recombinases, and polymerases, indicates that FFC promotes resistance gene transfer and FFC efflux[14]. FFC treatment increases ARG abundance in bacterial communities. High levels of ARGs persist even after stopping FFC use.

Eggs and egg products are recognized as important vehicles for foodborne *Salmonella* transmission, which can contaminate eggs through both vertical transmission from infected reproductive tissues and horizontal contamination from the production or handling environment. Therefore, contaminated eggs remain a relevant source of human salmonellosis and an important target for food-safety control[15]. Reported cases of *Salmonella* Typhimurium infection caused by egg consumption are gradually increasing[16]. The public-health concern is further amplified by the occurrence of antimicrobial-resistant *Salmonella* in poultry and egg samples. Previous studies have reported *Salmonella* Enteritidis contamination in poultry and egg samples, with isolates showing antimicrobial resistance and multidrug resistance phenotypes. A global review also emphasized that antimicrobial resistance in *Salmonella* from poultry and poultry-derived foods, including eggs, is a persistent concern for food safety and public health. These findings suggest that eggs may simultaneously serve as a vehicle for pathogenic bacteria and a potential route of exposure to antimicrobial residues.

In vitro, continuous induction with FFC, even at sub-inhibitory concentration stress, prompts *Salmonella* to develop specific resistance against FFC[17] and drives the bacteria towards a multidrug-resistant phenotype. This occurs through complex molecular mechanisms. It is accompanied by synergistic expression and enrichment of non-targeted ARGs. Continuous FFC induction is an important driving force for broad-spectrum multidrug resistance target mutations and acquisitions. Studies found that FFC pressure directly selects specific mutant strains carrying the ribosomal methyltransferase gene (*cfr*) and the ribosomal protection protein genes (*optrA*, *fexA*)[18]. The expression of the *cfr* gene confers *Salmonella* resistance to amphenicols and mediates the classic $PhLOPS_A$ resistance phenotype[19] (exhibits extensive cross-resistance to pleuromutilins, lincosamides, and streptogramin A), while affects vital human reserve drugs like oxazolidinones (e.g., linezolid).

Therefore, this study aimed to evaluate whether dietary exposure to FFC residues associated with eggs can promote antimicrobial resistance in *Salmonella* and alter the intestinal resistome. First, an egg-derived FFC-susceptible *Salmonella* Typhimurium isolate was subjected to long-term *in vitro* induction to determine whether FFC pressure could generate a stable resistant phenotype and cross-resistance to other antibiotics. Second, a mouse dietary exposure model was established to compare the effects of egg matrix alone, free FFC, and egg-associated FFC on the gut microbiota and ARG profiles. Third, a two-week withdrawal experiment was performed to assess the early self-recovery potential of the intestinal microbiota and resistome after cessation of exposure. This integrated design provides experimental evidence for evaluating the food-chain risk of FFC residues in eggs.

2 Materials and methods

2.1 Sample preparation and experimental design

Egg-derived *Salmonella* was isolated from egg samples obtained from the Hao You Duo supermarket in Yangling, Shaanxi Province, and identified as *Salmonella* Typhimurium. FFC-susceptible *Salmonella* (with determined minimum inhibitory concentration (MIC)) was inoculated into solid LB medium. It was cultured at 37 °C for 18–24 h. The induction started from 1/2 MIC (8 mg/L), and concentrations were raised stepwise. After reaching 16 μg/mL, subsequent passages increased the concentration by 8 μg/mL per passage (e.g., 16, 24, 32, and 40 μg/mL, etc.) until resistant isolates with stable elevated MICs were obtained. Each passage was performed in duplicate. The change in MIC before and after drug induction was recorded. A change greater than or equal to 4 times the original MIC was considered significant.

The 80 male C57BL/6J mice at 3 weeks of age were purchased from the Huafukang Biotechnology Co. (Beijing, China). The experimental mice were maintained under controlled conditions as follows: (22 ± 2) °C with (50 ± 15)% relative humidity and a 12 h/12 h light-dark cycle. Fecal samples were collected one day before sacrifice. Mice were placed in sterile beakers to allow spontaneous defecation. The feces were collected with sterile forceps to 1.5 mL sterile Eppendorf tubes and frozen at −80 °C. All experimental procedures were performed following the Guide for the Care and Use of Laboratory Animals (8th edition; ISBN-10: 0-309-15396-4), and the animal protocol was approved by the animal ethics committee of Northwest A&F University (IACUC2025-0328).

All mice were allowed to eat and drink freely, among the intervention groups (CCK: oral gavage of normal saline solution for 8 weeks; CEGG: oral gavage of whole egg (2.47 g/kg bw) for 8 weeks; CEF: oral gavage of whole egg (2.47 g/kg bw) and FFC (37 μg/kg bw) for 8 weeks; and CFFC: oral gavage of FFC (37 μg/kg bw) for 8 weeks) and self-recovery groups (HCK: oral gavage of normal saline solution for 10 weeks; HEGG: oral gavage of whole eggs (2.47 g/kg bw) for 8 weeks and then gavage stopped for 2 weeks; HEF: oral gavage of whole eggs (2.47 g/kg bw) and FFC (37 μg/kg bw) for 8 weeks and then gavage stopped for 2 weeks; HFFC: oral gavage of FFC (37 μg/kg bw) for 8 weeks and then gavage stopped for 2 weeks).

A two-week withdrawal period was selected to evaluate the early self-recovery capacity of the gut microbiota and resistome after cessation of FFC exposure. This period was not intended to demonstrate complete microbiota restoration, but to capture whether the major exposure-induced changes persisted after a short drug-free interval. Previous mouse studies have shown that antibiotic exposure can induce gut microbiota dysbiosis and host metabolic alterations that persist after antibiotic withdrawal[20]. Recovery after antibiotics is also strongly influenced by host diet, microbial community context and environmental reservoirs. In addition, studies on antibiotic-induced resistome disruption have shown that ARG profiles may remain altered after antibiotic cessation and may require additional interventions, such as prebiotics, probiotics or fecal microbiota transplantation, to accelerate recovery[21]. Therefore, the two-week withdrawal period was used as an early recovery window suitable for assessing the persistence of FFC-induced resistome alterations.

In this experiment, the correction coefficient method was used to calculate the intake conversion of eggs and FFC in humans and mice. The method was estimated by dividing the average body weight (kg) of the species by its body surface area (m^2). The body surface area correction factor (K_m) value of 60 kg adults was 37, and the K_m value of 20 g mice was 3. The K_m value of 60 kg adults and the K_m value of 20 g mice were used to estimate the intake of mice: mouse intake = human intake × 37/3. According to the National Food and Drug Administration's risk assessment of daily intake of FFC, adults (calculated as 60 kg) take 0–180 μg of FFC (acceptable daily intake is 0–3 μg/kg bw) from the diet every day. Under normal circumstances, there is no risk, but long-term consumption of eggs with excessive FFC residues will cause certain harm to human health. In this experiment, 60 kg of adult daily intake of 180 μg FFC, 240 g of fresh eggs converted to 20 g of mice daily intake of 0.74 μg FFC and 98.6 mg of freeze-dried eggs.

2.2 Real-time quantitative polymerase chain reaction (real-time PCR) analysis

The mRNA expression levels were quantified using a QuantStudio™ 5 system following the manufacturer's instructions with the 2× SYBR Green qPCR Master Mix (US Everbright, Suzhou, China). The total reaction volume was 20 μL, containing 10 μL of 2× SYBR Green qPCR Master Mix, 2 μL of cDNA template of 5 ng/μL, 1 μL each of forward and reverse primers of 10 μmol/L, and 6 μL of diethyl pyrocarbonate-treated RNase-free water. The PCR amplification program was performed according to the manufacturer's instructions. The 16S rRNA gene was used as the internal reference, and relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table 1.

2.3 Metagenomics

Genomic DNA was extracted from samples using DNA extraction kits provided by Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China). The integrity and purity of DNA were assessed by 1% agarose gel electrophoresis. DNA concentration and purity were also measured using Qubit 4.0 and NanoDrop One instruments (Thermo Fisher Scientific, Waltham, USA). DNA samples that met quality criteria were used for library preparation with the ALFA-SEQ DNA Library Prep kit (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China). Functional annotation of Unigenes was performed using the Comprehensive Antibiotic Resistance Database (https://arpcard.mcmaster.ca)[22].

2.4 Statistical analysis

The experiments were performed three times, and the results were presented as the mean ± standard deviation. Data were analyzed using SPSS V20 software. The two-tailed unpaired Student's *t*-test or one-way analysis of variance with Tukey's multiple comparison tests were performed to analyze statistical differences among the groups.

3 Results and discussion

3.1 *In vitro* resistance induction results

The parental egg-derived *Salmonella* Typhimurium isolate was susceptible to FFC, with an MIC of 8 mg/L. After continuous *in vitro* induction under gradually increasing FFC concentrations, the isolate acquired a stable resistant phenotype. The induced strain was further passaged for three generations in antibiotic-free medium to evaluate resistance stability. The final MIC of the induced strain reached ≥ 64 mg/L, indicating that continuous FFC exposure effectively promoted the development of FFC resistance in *Salmonella* (Table 2). These results suggest that FFC can act as a strong selective pressure for resistance evolution in foodborne *Salmonella*.

Table 1 Primer sequence.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
gyrA	AAATCGGCCCGTGTAGT	TGCCATACCCACGGCA
sulI	TGCAGGCTGGTGGTGGTTA	CGCGTGGGTGCGGACGT
tetA	GCTACATCCTGCTTGCCTTC	CATAGATCGCCGTGAAGAGG
cfr	GCAGGTTGGGAGTCATTTTG	ACGGTTGGCTAGAGCTTCAC
fexA	TCGCTGTTCTTGTGTTCGTC	ACAGCCCCATCAGAGTCATC
optrA	ACCACCTATCCTAAAGCGC	CTGATACGCTAAGTCAGCG
pexA	TGTTGTGGTTAATAACCGCAGCA	CACGGAAACCTTGTTACGACTTTC
floR	TCGGTCATTCACCACCGCAC	GCGGCCACGCTGTTCATGC
16S rDNA	CGGTGAATACGTTCYCGG	GGHTACCTTGTTACGACTT

Table 2 *In vitro* induction of *Salmonella* resistance by FFC.

Bacterial strain	Drug sensitive test MIC (mg/L)	
	Before induction	After induction
Salmonella	8 (sensitive)	64 (resistant)

3.2 Antimicrobial susceptibility changes of *Salmonella* after FFC induction

Compared with the susceptible parental strain, the FFC-induced resistant strain showed increased MIC not only to FFC but also to several non-target antibiotics, including tetracycline and cefoxitin. This result indicates that FFC-induced resistance was accompanied by a broader multidrug-resistance phenotype. Such cross-resistance may be associated with the activation of general resistance mechanisms, particularly efflux pumps and target protection systems. These phenotypic results were consistent with the increased abundance of multidrug-resistance-related gene families observed in the *in vivo* metagenomic analysis (Figures 1A–B).

3.3 Real-time PCR validation of *Salmonella* after FFC induction

Real-time PCR analysis was performed to further determine the molecular basis of the induced resistant phenotype. Compared with the susceptible strain, the FFC-induced resistant strain exhibited significantly higher expression levels of several resistance-related genes (Figure 1C). In particular, *fexA*, which encodes an efflux pump associated with chloramphenicol and FFC resistance, was markedly upregulated ($P < 0.000\ 1$). The expression levels of *tetA* and *optrA* were also significantly increased ($P < 0.05$ or $P < 0.01$). These results indicate that the FFC-induced multidrug-resistance phenotype was closely associated with enhanced efflux activity and ribosomal protection mechanisms.

Three specific genes showed no significant differences between the two strains: *floR* (mediating specific FFC resistance), *cfr* (a multidrug-resistant ribosomal methyltransferase), and *gyr* (associated with fluoroquinolone targets). This indicates that the multidrug resistance phenotype of the resistant strain is primarily driven by specific pathways, including the overexpression of *fexA* and *optrA*. These qualitative and quantitative *in vitro* data correlate well with the subsequent increase in the corresponding ARG families in the intestinal microbiome resistome.

3.4 Metagenomic analysis of ARGs

3.4.1 ARGs composition analysis

Differences in the average relative abundance of ARGs among the experimental groups were analyzed. The high-abundance resistance reservoir was primarily composed of four antibiotic classes: fluoroquinolones, tetracyclines, macrolides, and penams. Comparison between the CCK and CEF groups revealed an upregulation of total ARG abundance in the CEF group, predominantly in the β-lactam resistance dimension, including cephalosporins, cephamycins, and penams, indicating directed antibiotic selection pressure. In comparison with the CCK group, the CFFC group exhibited greater differences, with significantly increased total resistance levels. Specifically, the relative abundances of aminoglycosides, macrolides, and tetracyclines were markedly higher in the CFFC group. Further comparison of the CEF and CFFC groups showed that the CFFC group had significantly elevated relative abundances in peptide antibiotics, fluoroquinolones, and glycylcyclines. The HEF group accumulated significantly more ARGs in aminoglycosides and disinfecting agents/antiseptics compared to the CEF group, whereas the HFFC group displayed the largest differences relative to the CCK group, particularly in tetracyclines, rifamycins, and phenicols (Figures 2A–B).

Analysis of ARG family relative abundances indicated that the resistome was dominated by three major families: ABC antibiotic efflux pumps, RND antibiotic efflux pumps, and MFS antibiotic efflux pumps (Figures 2C–D). Compared with the CCK group, the CEF group exhibited increased abundances in two families, including ABC antibiotic efflux pump and RND antibiotic efflux pump. In the CCK versus CFFC comparison, the CFFC group showed significantly higher relative abundances of ABC and MFS efflux pumps, as well as the glycopeptide resistance gene cluster. Furthermore, compared with the CEF group, the CFFC group displayed upregulated abundances of the MATE transporter.

The self-recovery groups revealed that the intestinal resistome did not return uniformly to the control state after the two-week withdrawal period. HEGG was generally close to HCK, supporting the view that discontinuation of egg gavage alone did not result in persistent resistome disturbance. In contrast, HEF still retained higher abundances of several ARG categories than CEF, particularly aminoglycoside resistance genes and genes related to disinfecting agents and antiseptics, indicating that withdrawal after dietary FFC-residue exposure did not fully restore the resistome to the basal state. The most pronounced persistence was observed in HFFC. Compared with HCK, HFFC maintained elevated abundances of tetracycline, rifamycin and phenicol resistance genes, together with specific resistance determinants such as rifamycin-resistant *rpoB* and the vanS regulatory protein. The HEF group exhibited significant upregulation in RND efflux pumps and polymyxin resistance phosphoethanolamine transferase, while the HFFC group showed increased abundances of specific resistance

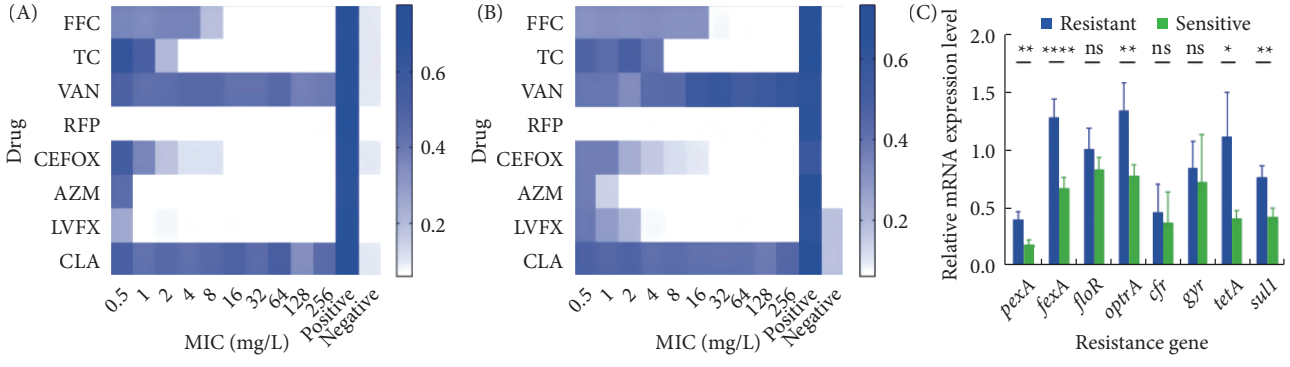

Figure 1 Drug susceptibility testing and real-time PCR results of *Salmonella* induced by FFC. (A) Heat map of susceptibility results for sensitive strains; (B) heat map of susceptibility results for resistant strains; (C) real-time PCR result graph. *$P < 0.05$; **$P < 0.01$; ****$P < 0.000\ 1$; ns means no significant differences between two groups. TC, tetracycline; VAN, vancomycin; RFP, rifampin; CEFOX, cefoxitin; AZM, azithromycin; LVFX, levofloxacin; CLA, clarithromycin.

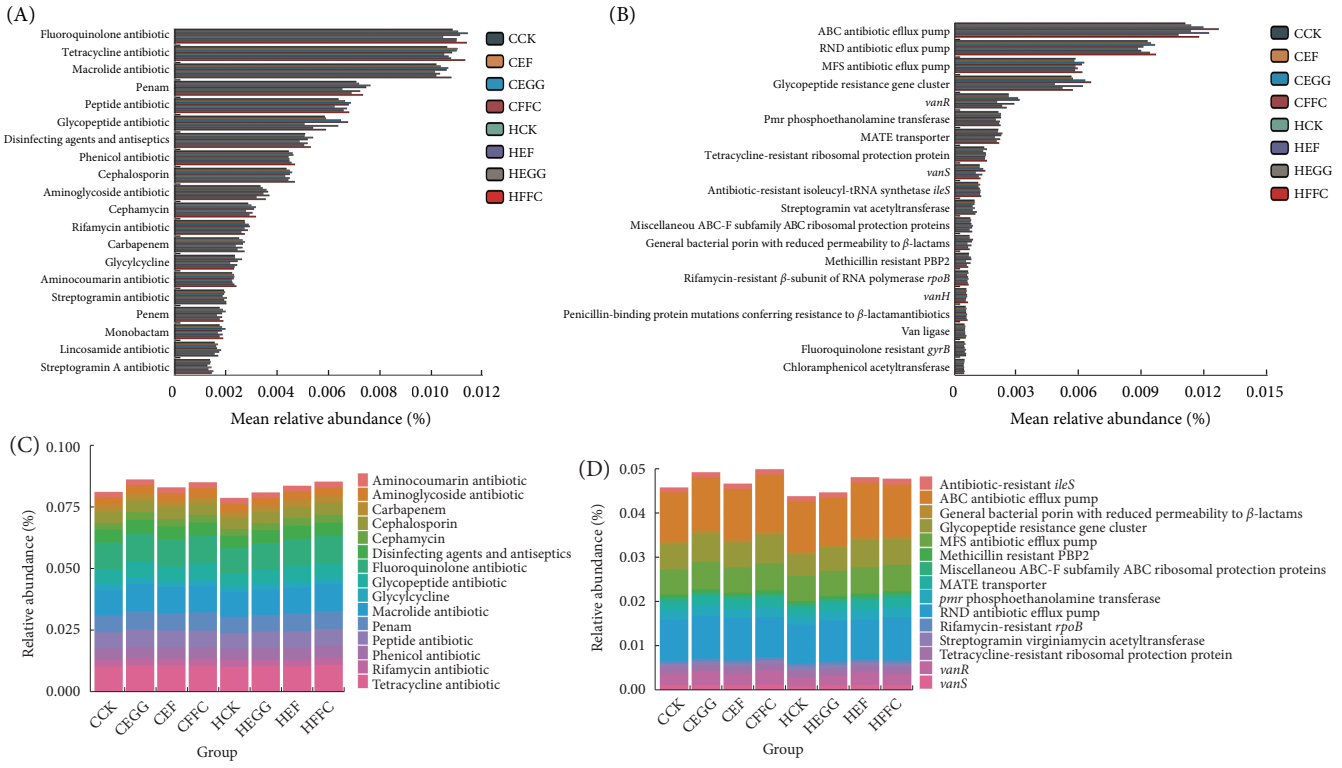

Figure 2 ARGs composition analysis. (A) Multigroup differential analysis plot of relative abundance of ARGs; (B) multigroup differential analysis plot of relative abundance of antibiotic resistance gene families; (C) composition analysis plot of relative abundance of ARGs; (D) composition analysis plot of relative abundance of antibiotic resistance gene families. MATE, multidrug and toxic compound extrusion; *rpoB*, β-subunit of RNA polymerase; *ileS*, isoleucyl-tRNA synthetase.

genes, including the rifamycin-resistant *rpoB* and the vanS protein, a regulator of glycopeptide resistance.

To clarify the baseline influence of the egg matrix, we further compared the CEGG group with the CCK group. Overall, the CEGG group showed a resistome profile closer to that of CCK than to those of the FFC-exposed groups. The relative abundances of the major ARG classes, including fluoroquinolone, tetracycline, macrolide and β-lactam resistance genes, did not show the broad enrichment pattern observed in CEF or CFFC. At the ARG-family level, the dominant efflux-related families, including ABC, RND and MFS antibiotic efflux pumps, remained relatively stable between CEGG and CCK, indicating that the pure egg matrix did not impose a strong antibiotic-like selective pressure on the intestinal resistome.

The difference between CFFC and CEF may be partly explained by the matrix-dependent bioavailability of FFC in the intestinal tract. Although both groups received the same nominal FFC dose, FFC in CFFC was administered as free drug, whereas FFC in CEF was delivered together with the whole-egg matrix. Previous studies on veterinary drug residues in eggs have shown that FFC and FFC amine can be deposited in both yolk and albumen, with residue depletion and persistence being strongly influenced by the physicochemical properties of the egg compartments, especially the lipid-rich yolk fraction[23]. Therefore, the egg matrix may alter the dissolution, release and intestinal accessibility of FFC. Compared with free FFC, matrix-associated FFC may produce a lower transient luminal concentration or a slower release profile, thereby reducing the immediate selective pressure on intestinal bacteria. This interpretation is consistent with our observation that CFFC induced stronger enrichment of peptide antibiotic, fluoroquinolone and glycylcycline resistance genes, as well as higher abundances of MATE transporters and tetracycline-resistant ribosomal protection proteins, than CEF. In contrast, CEF showed a more moderate resistome shift, suggesting that the egg matrix may partially buffer the ecological impact of FFC residues.

3.4.2 *Resistance mechanism analysis*

A relative abundance analysis of antibiotic resistance mechanisms was conducted based on metagenomic data (Figure 3). Two core mechanisms dominated the resistome: antibiotic efflux and antibiotic target alteration. Comparison between the CCK and CEF groups showed that the total abundance of resistance mechanisms in the CEF group tended to be higher than in the CCK group, although the distribution of major mechanisms remained largely consistent.

More pronounced differences were observed between the CCK and CFFC groups, with the CFFC group exhibiting a significant increase in total relative abundance, primarily driven by enhanced antibiotic efflux and target alteration mechanisms. Comparison of the CFFC and CEF groups revealed that the CFFC group had higher overall resistance mechanism abundance, with secondary mechanisms—including antibiotic inactivation and target replacement—also significantly elevated relative to the CEF group.

The HEF group displayed a slightly higher total abundance than the CEF group. Among all samples, the HFFC and CFFC groups maintained the highest total resistance mechanism abundances, with minimal differences between them and a high degree of overlap in specific resistance mechanisms.

3.4.3 *ARGs bacterial host analysis*

The relative abundance of ARG-hosting bacterial taxa was analyzed (Figure 4). Comparison between the CCK and CEF groups revealed upregulation of *Actinospica robiniae* and *Pseudomonas* in the CEF group.

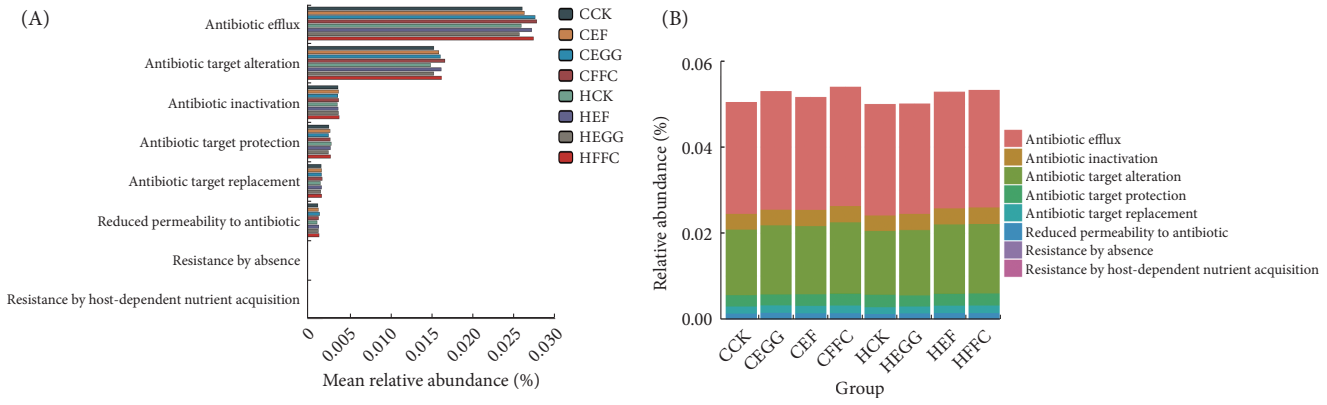

Figure 3 Resistance mechanism analysis. (A) Diagram of multi-group differential analysis of relative abundance of antibiotic resistance mechanisms; (B) composition analysis diagram of relative abundance of antibiotic resistance mechanisms.

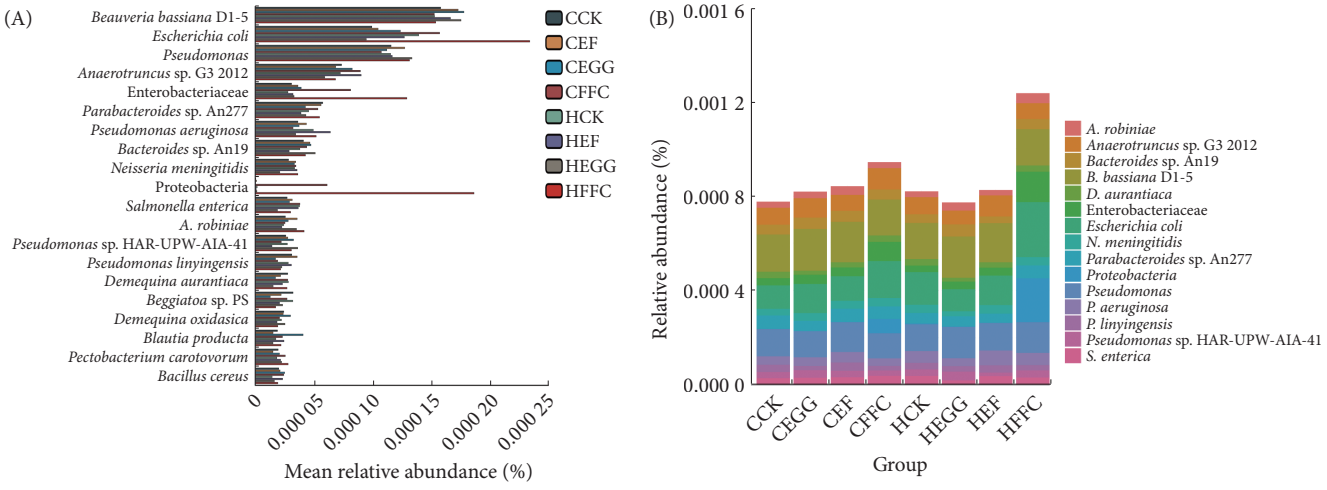

Figure 4 ARG bacterial host analysis. (A) Diagram of multi-group differential analysis of relative abundance of ARGs bacterial host; (B) composition analysis diagram of relative abundance of ARGs bacterial host.

In the comparison between the CFFC and CCK groups, several species exhibited significantly increased relative abundances in the CFFC group, including *S. enterica*, *Anaerotruncus* sp. G3 2012 and *Bacteroides* sp. An19.

When comparing the CFFC and CEF groups, the CFFC group displayed greater species complexity, with notably higher relative abundance of *D. aurantiaca* than in the CEF group. The HEF and CEF groups showed similar microbial structures, although the relative abundance of *Anaerotruncus* sp. G3 2012 was slightly elevated in the HEF group.

ARG-host analysis further showed that Proteobacteria, Enterobacteriaceae and *E. coli* remained enriched in HFFC, suggesting that free FFC exposure may promote a more stable dysbiotic and resistance-associated community. These findings indicate that cessation of FFC exposure is not sufficient for complete short-term recovery of the intestinal resistome. This is consistent with previous mouse studies showing that bacterial load may recover within approximately one week after antibiotic withdrawal, whereas microbial community composition, diversity and functional profiles can remain altered for longer periods. In the HFFC group, the relative abundances of Proteobacteria, Enterobacteriaceae, and *E. coli* increased significantly, whereas *Parabacteroides* sp. An277 and *N. meningitidis* showed decreased relative abundances.

Similarly, ARG-host analysis showed no obvious expansion of Proteobacteria, Enterobacteriaceae or *E. coli* in CEGG compared with CCK. These findings suggest that the egg matrix alone had a limited effect on gut resistome structure under the present experimental conditions, and that the resistome expansion observed in CEF was mainly associated with FFC exposure rather than egg intake per se. Nevertheless, the CEGG group remains essential because it provides the baseline matrix control required to interpret the biological effect of FFC residues delivered through eggs.

4 Conclusion

This study combined an *in vitro* induction model with metagenomic sequencing to investigate how FFC promotes multidrug resistance in *Salmonella* and alters the intestinal microecology. Under FFC selection pressure, susceptible *Salmonella* rapidly evolved into resistant strains (MIC ≥ 64 mg/L), accompanied by significant upregulation of efflux pump genes (e.g., *fexA* and *tetA*) and the ribosomal protection gene *optrA*. These changes contributed to cross-resistance to non-target antibiotics, including quinolones and *β*-lactams.

The *in vivo* metagenomic results further showed that FFC exposure reshaped the intestinal resistome. In the CFFC and HFFC groups, resistance profiles were mainly characterized by increased abundance of antibiotic efflux systems, including ABC, RND, and MFS transporters, as well as target modification mechanisms. FFC exposure also disturbed intestinal microbial composition and

enriched ARG-associated opportunistic taxa, particularly Proteobacteria and Enterobacteriaceae.

Although metagenomic sequencing revealed ARG enrichment, it did not determine whether these genes were located on transferable plasmids or other MGEs, nor did it experimentally verify their horizontal transfer potential. In addition, the two-week withdrawal period only reflects short-term recovery and may be insufficient to evaluate long-term restoration of the gut microbiota and resistome. Because this study was conducted in mice, extrapolation to human dietary exposure should be made cautiously. In future studies, longer withdrawal periods are also needed to assess resistome recovery. In addition, mitigation strategies such as dietary fiber supplementation, probiotics, and microbiota restoration should be evaluated.

Conflict of interest

The authors declare no conflicting financial interests.

Acknowledgements

This work was supported by the Risk Assessment of Agricultural Product Quality and Safety Project (K4050423407). The authors would like to thank the instrument sharing platform of the College of Food Science & Engineering of Northwest A&F University, for the assistance in the fluorescence microscopy measurements.

Author contributions

Yan Gong: Conceptualization, methodology, validation, formal analysis, investigation, data curation, and writing-original draft. Wenduo Han: Methodology, investigation and formal analysis. Jie Bai: Methodology and software. Xuebo Liu: Conceptualization and supervision. Xiang Duan: Conceptualization, formal analysis, funding acquisition, writing-review & editing, and supervision.

References

[1] Y. Li, W. Cao, S. Liang, et al., Metagenomic characterization of bacterial community and antibiotic resistance genes in representative ready-to-eat food in southern China, Sci. Rep. 10 (2020) 15175. https://doi.org/10.1038/s41598-020-72620-4.

[2] Z. Ma, L. Lin, X. Yang, et al., Effects of florfenicol exposure during early life on toxicity, gut microbiota, and fecal metabolome in SD rats, Ecotoxicol. Environ. Saf. 228 (2021) 113038. https://doi.org/10.1016/j.ecoenv.2021.113038.

[3] M. E. Velazquez-Meza, M. Galarde-López, B. Carrillo-Quintero, et al., Antimicrobial resistance: One Health approach, Vet. World 15 (2022) 743–749. https://doi.org/10.14202/vetworld.2022.743-749.

[4] Y. Guo, G. Song, M. Sun, et al., Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*, Front. Cell. Infect. Microbiol. 10 (2020) 207. https://doi.org/10.3389/fcimb.2020.00207.

[5] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat. Rev. Microbiol. 19 (2021) 55–71. https://doi.org/10.1038/s41579-020-0433-9.

[6] K. Ma, Y. Feng, L. Liu, et al., High prevalence of *cfr* and *optrA* in *Salmonella* isolated from diverse sources in China, Poult. Sci. 101 (2022) 102146. https://doi.org/10.1016/j.psj.2022.102146.

[7] C. Crowe-McAuliffe, V. Murina, K. J. Turnbull, et al., Structural basis for antibiotic resistance mediated by the multiresistance gene *optrA*, Proc. Natl. Acad. Sci. USA 118 (2021) e2026355118. https://doi.org/10.1073/pnas.2026355118.

[8] D. B. Holman, K. E. Gzyl, A. Kommadath, Florfenicol administration in piglets co-selects for multiple antimicrobial resistance genes, mSystems 9 (2024) e01250-24. https://doi.org/10.1128/msystems.01250-24.

[9] X. Guo, Z. Qian, Q. Pan, et al., Effects of florfenicol on intestinal histology, apoptosis and gut microbiota of Chinese mitten crab (*Eriocheir sinensis*), Int. J. Mol. Sci. 24 (2023) 4412. https://doi.org/10.3390/ijms24054412.

[10] T. Zhang, Y. Ding, J. Peng, et al., Effects of broad-spectrum antibiotic (florfenicol) on resistance genes and bacterial community structure of water and sediments in an aquatic microcosm model, Antibiotics 11 (2022) 1299. https://doi.org/10.3390/antibiotics11091299.

[11] Z. Zhang, Y. Li, M. Hu, et al., Comparative transcriptome profiling reveals a mechanism of *Streptococcus agalactiae* resistance to florfenicol, Microb. Pathog. 142 (2020) 104098. https://doi.org/10.1016/j.micpath.2020.104098.

[12] R. S. McInnes, G. E. McCallum, L. E. Lamberte, et al., Horizontal transfer of antibiotic resistance genes in the human gut microbiome, Curr. Opin. Microbiol. 53 (2020) 35–43. https://doi.org/10.1016/j.mib.2019.10.003.

[13] H. Li, Y. Qi, J. Li, et al., Antibiotic-induced gut dysbiosis promotes metabolic syndrome via altered bile acid metabolism, Gut Microbes 15 (2023) 2176974. https://doi.org/10.1080/19490976.2023.2176974.

[14] S. R. Partridge, S. M. Kwong, N. Firth, et al., Mobile genetic elements associated with antimicrobial resistance, Clin. Microbiol. Rev. 31 (2018) e00088-17. https://doi.org/10.1128/CMR.00088-17.

[15] L. Hurtado, C. D. Miranda, R. Rojas, et al., Live feeds used in the larval culture of red cusk eel, *Genypterus chilensis*, carry high levels of antimicrobial-resistant bacteria and antibiotic-resistance genes (ARGs), Animals 10 (2020) 505. https://doi.org/10.3390/ani10030505.

[16] C. Jans, E. Sarno, L. Collineau, et al., The impact of food on the human gut resistome: a review, Front. Microbiol. 13 (2022) 1035541. https://doi.org/10.3389/fmicb.2022.1035541.

[17] M. Levé, P. Manghi, M. Bredon, et al., Metabolomics and metagenomics in mice reveal the role of the gut microbiota in tryptophan metabolism, iScience 28 (2025) 113751. https://doi.org/10.1016/j.isci.2025.113751.

[18] R. Li, H. Wang, Q. Shi, et al., Effects of oral florfenicol and azithromycin on gut microbiota and adipogenesis in mice, PLoS ONE 12 (2017) e0181690. https://doi.org/10.1371/journal.pone.0181690.

[19] W. Li, Y. Li, X. Jiang, et al., Compound ammonium glycyrrhizin protects hepatocytes from injury induced by lipopolysaccharide/florfenicol through a mitochondrial pathway, Molecules 23 (2018) 2378. https://doi.org/10.3390/molecules23092378.

[20] X. Li, H. Chen, H. Yue, et al., Depletion of florfenicol and florfenicol amine in eggs of laying hens and growing pullets after oral administration, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 37 (2020) 1711–1722. https://doi.org/10.1080/19440049.2020.1780190.

[21] S. Acosta, G. Herrera, R. Ortiz, et al., Antibiotic treatment induces long-lasting effects on gut microbiota and the enteric nervous system in mice, Antibiotics 12 (2023) 1000. https://doi.org/10.3390/antibiotics12061000.

[22] B. P. Alcock, A. R. Raphenya, T. T. Lau, et al., CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database, Nucleic Acids Res. 48 (2020) D517–D525. https://doi.org/10.1093/nar/gkz935.

[23] J. Li, Y. Li, J. Wang, et al., The prolonged disruption of a single-course amoxicillin on mice gut microbiota and resistome, and recovery by inulin, *Bifidobacterium longum* and fecal microbiota transplantation, Environ. Pollut. 265 (2020) 114651. https://doi.org/10.1016/j.envpol.2020.114651.