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PHARMACODYNAMIC ANALYSIS OF ENROFLOXACIN RESISTANCE IN BROILER APEC: A TARGETED REVIEW OF VALIDATED NON-ANTIBIOTIC CONTROL STRATEGIES

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Abstract

Avian pathogenic *E. coli* (APEC) is the causative agent of colibacillosis, which can cause systemic and localized infections in poultry; however, multiple serotypes contribute to the development of colibacillosis disease. Enrofloxacin is used as a therapeutic agent to kill APEC; moreover, enrofloxacin is a broad-spectrum antibiotic that can be administered orally, intramuscularly, and subcutaneously. The pharmacokinetics-pharmacodynamics (PK/PD) parameters can play an important role in determining its efficacy, and the performance of the broilers; however, killing of bacteria also depends upon the dose concentration of enrofloxacin. Multiple challenges are associated with enrofloxacin, such as resistance issues, which can be due to misuse and administration of the drug via drinking behaviour and drug residue in the edible part of the chicken; however, its misuse leads to mutation in the genes of DNA gyrase and Topo IV, increasing the potential for resistance. Due to emerging resistance, alternative approaches such as nanoparticles, synbiotic combinations, phytochemicals, and essential oils can be used that may reduce bacterial growth and thus decrease the dependence on enrofloxacin. However, this review focuses on the role of enrofloxacin, its PK/PD model, the accelerating resistance, and emerging non-antibiotic control.

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INTRODUCTION

Poultry is one of the rapidly growing sectors worldwide over the years, with a substantial growth rate. (Erdcaw and Beyene, 2022; Rashid and Shnawa, 2024; Tariq et al., 2025; Afzal et al., 2026). As the world population is growing, the poultry producers have more opportunities to capitalize on this industry in both developed and developing countries (George and George, 2023; Ahmad et al., 2024, Tariq et al., 2026). But as demand increases in the poultry industry, challenges also expand (Yajie et al., 2023), because with the development of poultry sectors, the exacerbation of poultry disease is leading to a decrease in their productivity (Afzal et al., 2025; Tariq et al., 2026). These diseases are threats to poultry farmers because they damage the economics and cause losses; moreover, among these diseases, colibacillosis is one of the top persistent diseases ascribed to *Escherichia coli (E. coli)* (Kika et al., 2023; Tariq et al., 2025; ABBAS et al., 2025, Pervaiz et al., 2025). It is a wide-ranging term that expresses the group of diseases provoked by various pathogenic strains of *E. coli* (Okpalaji et al., 2025). The ferocity and occurrence of this disease depend on multiple factors, such as bacterial strain characteristics, external environmental or surrounding conditions, and the immune system of the host. The economic burden due to this disease is considerable, imposed in such a way that it reduces production rates, culls infected birds, and increases medication expenditures (Kika et al., 2023; Ashraf et al., 2025). All age groups of chickens have been affected by this disease, but adult layers are most susceptible, moreover, *E. coli* mostly resides in the intestine of animals as well as poultry. *E. coli* is observed in chicken feces and is more frequently found in feces than other bacteria; moreover, the microbiota residing in the caecum of healthy chickens carry almost 10 to 15% of the O-serotype of *E. coli* that is also seen in colibacillosis lesions (Lutful, 2010). The etiological agent of systemic or localized infections in colibacillosis is avian pathogenic *E. coli* (APEC), and it is a subpathotype of extraintestinal pathogenic *E. coli* (ExPEC) (Christensen et al., 2021; Shehata and Hafez, 2024; Mehat et al., 2021). The signs and symptoms of this disease can vary widely from acute respiratory or intestinal illness to severe systemic disease that can cause extreme sickness and mortality (Kika et al., 2023; Serbessa et al., 2023). The localized infections of avian colibacillosis are

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expressed as cellulitis, peritonitis, yolk sac infection, and inflammation of the fallopian tube, while systemic infections are colisepticemia, coligranuloma, pericarditis, air sac inflammation, and arthritis (Joseph et al., 2023; Ahmed et al., 2025).

Colibacillosis, the disease, is driven by the prevailing strain known as strain O of APEC. Further, *E. coli* is a gram-negative, non-staining, non-acid-fast bacterium. Moreover, it is rod-shaped; various strains are often fimbriated and motile due to the peritrichous flagella that belong to the *Enterobacteriaceae* family. The various types of antigens can differentiate the serotypes of *E. coli*, such as flagellar (H), somatic (O), and occasionally capsular (K) antigens (Robi et al., 2024). Virulence factors that are present on the bacterial strains allow them to inhabit the mucosal surfaces of birds and cause the development of diseases. Multiple serotypes of APEC are linked with colibacillosis, but 3 major serotypes that cause almost 80% of colibacillosis are O1, O2, and O78 (Tian et al., 2025). A research group reported in 2021 that the most common APEC strains are O78, O24, O25, O2, and O86; moreover, recent studies reveal that O25, O8, and O15 serogroups are clinically isolated and contribute to the disease outbreak. Moreover, a statistical analysis of 189 APEC found unusual serogroups such as O117, O127, and O65 that are also causative agents of disease (Runcharoon et al., 2025). The infection of avian colibacillosis in domesticated birds is also commonly linked to some strains of serogroups O1:K1, O78:K80, and O2:K1 (2- Filali E).

Biosecurity and sanitation procedures can fail to eradicate APEC strains because of the ubiquitous nature and commensal relationship of *E. coli* with the intestinal microbiota of birds. Due to the genetic diversity of *E. coli* strains, the effect of vaccination is also decreasing; however, there is an antibiotic option that can be used to treat this disease to reduce mortalities. The most common classes of antibiotics are fluoroquinolones, β-lactams, tetracyclines, sulphonamides, and aminoglycosides, typically administered orally and frequently used against colibacillosis. Additionally, overuse of antibiotics can cause antimicrobial resistance (AMR), which is becoming a global challenge to treat colibacillosis and limit its use in poultry, making treatment challenging (Vougat et al., 2025). A study published in 2025 suggested some protective antibiotics against colibacillosis that

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reduced mortality, which included doxycycline, which is the most effective, then spectinomycin and enrofloxacin, which have strong effects, while flumequine and oxytetracycline have moderate effects, and combination therapy of lincomycin and spectinomycin also has a good effect as well, but some more studies need to confirm it confidently with the same age, administration route, breed and rate of dose, moreover, this study also focuses on enrofloxacin's effective use via drinking administration and its broad-spectrum nature. Additionally, enrofloxacin is classified as a category B drug by the European Medicines Agency (EMA), meaning it can be safe only if it is used correctly (right duration, administration, and dose) (Vougat et al., 2025).

Enrofloxacin belongs to the class of fluoroquinolones that are 2nd-generation fluoroquinolones and potent agents and are widely used in veterinary medicine against both gram-positive and gram-negative bacteria. This compound acts as a chemotherapeutic agent, and in 1983, it was synthesized for the 1st time from nalidixic acid. Moreover, in 1991, the first enrofloxacin was produced as a medicinal product, such as the oral form of the drug for poultry under the brand name Baytril®, and currently, it is available in both tablet and injectable forms authorized by the EMA (Grabowski et al., 2022). But now resistance has been reported against enrofloxacin due to unnecessary use, such as administration of the drug without checks and balances, causing treatment failure as well as health concerns due to the zoonotic importance of APEC strains (Nechypurenko et al., 2024; Durrani et al., 2024). Resistance is also accelerated due to different APEC strains; sometimes a specific drug can be effective against specific APEC strains, but not all strains. Moreover, when this specific drug is repeatedly used against APEC strains that may be less sensitive to that drug, it may cause resistance, so bacterial strains can be cultured, and an antibiotic susceptibility test (AST) can be used before the drug is applied for a better result. Multiple mechanisms are included to drive the resistance, such as quinolone resistance-determining regions (QRDR), activation of efflux pumps, and plasmid-mediated quinolone resistance genes (PMQR). These limitations are restricting the enrofloxacin use, emphasizing the necessity of such strategies that bypass these challenges; resultantly, a focus on the novel, eco-friendly and precision-guided approaches such as nanoparticles, phytochemicals,

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essential oils, synbiotic combinations, probiotics, vaccination against APEC-specific strains and phage therapy has been introduced in the market as a treatment strategy for effective control of colibacillosis induced by APEC.

This review particularly stresses an inclusive perspective on colibacillosis disease and particularly emphasizes the role of enrofloxacin, its mechanism of action, its efficacy, its challenges, and future alternatives.

Pathogenesis of APEC

Colibacillosis is caused by different pathogenic and virulence factors present on APEC, and infection of APEC may occur through the cloacal, oral, and nasal paths (Yu et al., 2025). The primary site of infection for APEC is the respiratory tract, from which it can spread to various organs (Li et al., 2025). The exceptional strategies employed by pathogenic bacteria include the adhesion system, which enables them to adhere to host cells. Colonization typically occurs in the mucosal membrane of the reproductive, gastrointestinal, and respiratory tracts without giving rise to disease; subsequently, invasion and replication occur (Hu et al., 2022). The bacterial transmission occurs through pathogenic eggs and the faecal-oral route, while the lungs are the primary infection area where bacteria enter the blood flow. The bacterial colonization is very crucial in the respiratory tract, which is facilitated by many virulence factors; after this, it can spread to many organs, causing systemic infection and thus organ damage (Nawaz et al., 2024).

The virulence factors that facilitated this pathogenesis are the following:

APEC Adhesions, Invasions protein and Iron Acquisition Systems

Cell-surface element of bacteria that helps in adherence to other surfaces or the epithelial membrane of the host cell during the earliest steps of APEC infections (Moxley, 2022). Adhesion plays a vital role in bacterial pathogenesis. Various types of adherence molecules are 1 fimbriae, S fimbriae, P fimbriae, outer membrane protein, flagella, curli, non-fimbrial, temperature-sensitive hemagglutinin, and atypical adhesins (Desvaux et al., 2020; Hu et al., 2022). Invasions are proteins that facilitate

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the entry of bacteria into the host; therefore, many genes, such as *ibe*A, *ibe*B, and *tia, encode* this protein and thus contribute to biofilm formation, resistance to oxidative stress, colonization, and division in the host (Kathayat et al., 2021). Iron acquisition system act as operating system that ensures the acquisition of iron that is essential for the growth and multiplication of APEC in the host. Multiple siderophores are produced by bacteria, including salmochelin, yersiniabactin, and aerobactin, accompanying some specific transporters that sequester the iron from fluids of the host body; moreover, a high number of the aerobactin Fe acquisition system is found in the APEC strains in contrast to the non-pathogenic strains (Kuznetsova et al., 2025).

APEC Protectins and pathogenic Toxins

Protectins are protein that protects in stress conditions against the host immune system, including outer membrane proteins (OMPs), capsules, and lipopolysaccharide (LPS). Moreover, capsules, a virulence factor, play a key role in septicemia, responsible for the transmission of APEC into the blood (Khairullah et al., 2024; Pokharel et al., 2023). ColV plasmid carries an *iss* gene that plays a crucial role in bacterial serum survival (Reid et al., 2025). Moreover, protectins also interpose in the bacterial adhesion, invasion, colonization, and multiplication in the host body and thus ensure the intracellular survival. However, another virulence factor that enhances the invading ability of the bacteria to damage the host cells is assisted by the biological poisons called toxins (Idris, 2024). Multiple types of toxins are cytotoxic necrotizing factor 1 (CNF1), various haemolysins, and vacuolating autotransporter toxin (Vat). Several genes coding for these multiple toxins (Hu et al., 2022; Carlini et al., 2021). Some other contributing virulence factors are the quorum-sensing (QS) system, two-component systems, transcriptional regulators, metabolism-associated genes, and secretion systems, which facilitate different stages of pathogenic bacterial infection, such as adhesion, colonization, and invasion. Moreover, interaction among bacteria, bypassing of host immune defenses, is also done by these virulence factors, which assist in promoting the disease in the host

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cell (Kathayat et al., 2021; Lamaudière et al., 2023). APEC pathogenesis in different organs is shown in Figure 1.

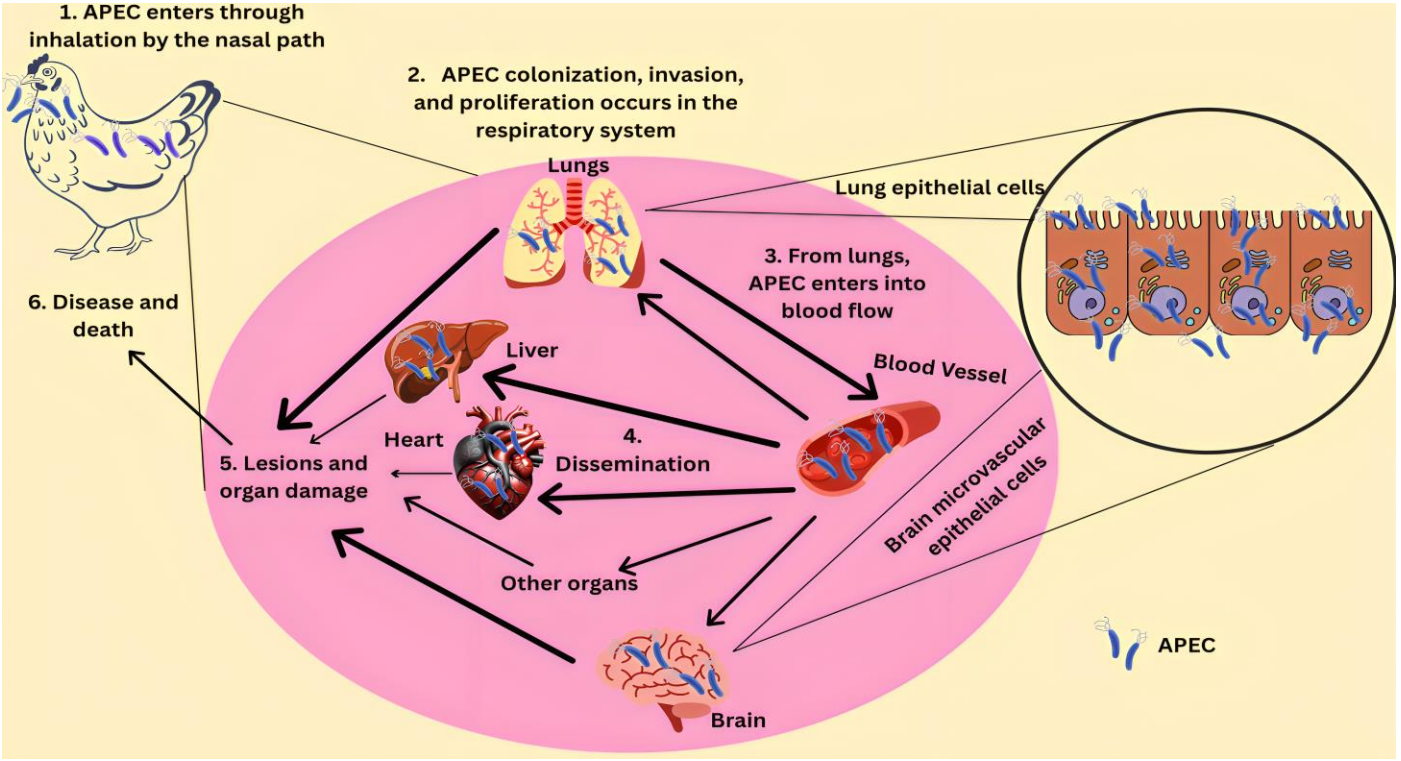

Figure 1. Dissemination of APEC infection from lungs to other organs.

Pharmacokinetics-pharmacodynamics (PK/PD) model

The term 'pharmacokinetics' describes the time from drug administration to drug elimination from the body; moreover, it is administered orally, intramuscularly, and subcutaneously based on species. However, it has fast absorption and peak bioavailability (Falahatipour et al., 2025), and the efficacy of the drug is based on distribution, especially the unmetabolized form of the drug in the blood and tissues. The plasma protein interacting ability of enrofloxacin is moderate, so a huge part of the drug is free, which is pharmacologically active; thus, the volume of distribution of the drug (Vd) in organs of different animals is different, such as the Vd in the heart, lung, liver, spleen and other organs of broiler chickens, which is 5.07 (L/kg). The primary metabolite of enrofloxacin is ciprofloxacin, but

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many other metabolites of enrofloxacin are inactive. In chickens, the conversion of enrofloxacin into ciprofloxacin is lower than in mammals; thus, the main circulating drug is enrofloxacin, with a small portion of ciprofloxacin acting as an antibacterial activity, and the enrofloxacin elimination also varies in different species. If enrofloxacin is administered intravenously, the half-life of drug elimination in broilers is 12.84 h, while other animals also have different half-lives. Ciprofloxacin follows the renal as well as hepatic routes, while enrofloxacin only follows the renal pathway for elimination (Grabowski et al., 2022).

Enrofloxacin depends upon concentration; a higher concentration of the drug produces rapid and immense killing against the E. coli strains. In pharmacodynamics, the efficacy of a drug is a big challenge due to antimicrobial resistance (AMR). Efficacy means the drug reaches the infection site and maintains its concentration that kills or inhibits bacteria (Abebe and Birhanu, 2023). There are two primary PK/PD parameters for all fluoroquinolones, such as enrofloxacin:

AUC_{0-24}/MIC ratio: AUC refers to the area under the curve; it is also described by the area below the plasma concentration versus the time curve to MIC, which represents the minimum inhibitory concentration. However, to check enrofloxacin efficacy, this is the crucial parameter that describes if a higher value of AUC_{0-24}/MIC means better clinical outcomes and low-level resistance. A study on the PK/PD model of enrofloxacin in broilers administered orally indicated that the AUC_{0-24}/MIC required for killing bacteria was 19.32, 32.15, and 23.41 in blood, liver, and lungs, respectively, when the enrofloxacin dose is administered alone; however, as a combined dose of enrofloxacin and its primary metabolite, ciprofloxacin, the AUC_{0-24}/MIC was reported to be 21.29, 41.68, and 27.65 in blood, liver, and lungs, respectively, while the elimination effect was reported to be even higher exposure, such as 32.13, 58.52, and 46.22 in blood, liver, and lungs, respectively (Xiao et al., 2018).

C_{max}/MIC ratio: It is the ratio between maximum plasma concentration and minimum inhibitory concentration, and this is also a good factor to decide the efficacy of enrofloxacin, like other drugs. Moreover, it indicates that the drug has reached a high enough maximum concentration to kill the

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bacteria rapidly, thus leading to minimizing the resistant bacterial strains. However, the Cmax/MIC of ≥ 8–10 is often associated with a predictive target to kill the bacteria to achieve maximum clinical outcomes (Xiao et al., 2018). The pharmacokinetics of enrofloxacin are shown in Figure 2.

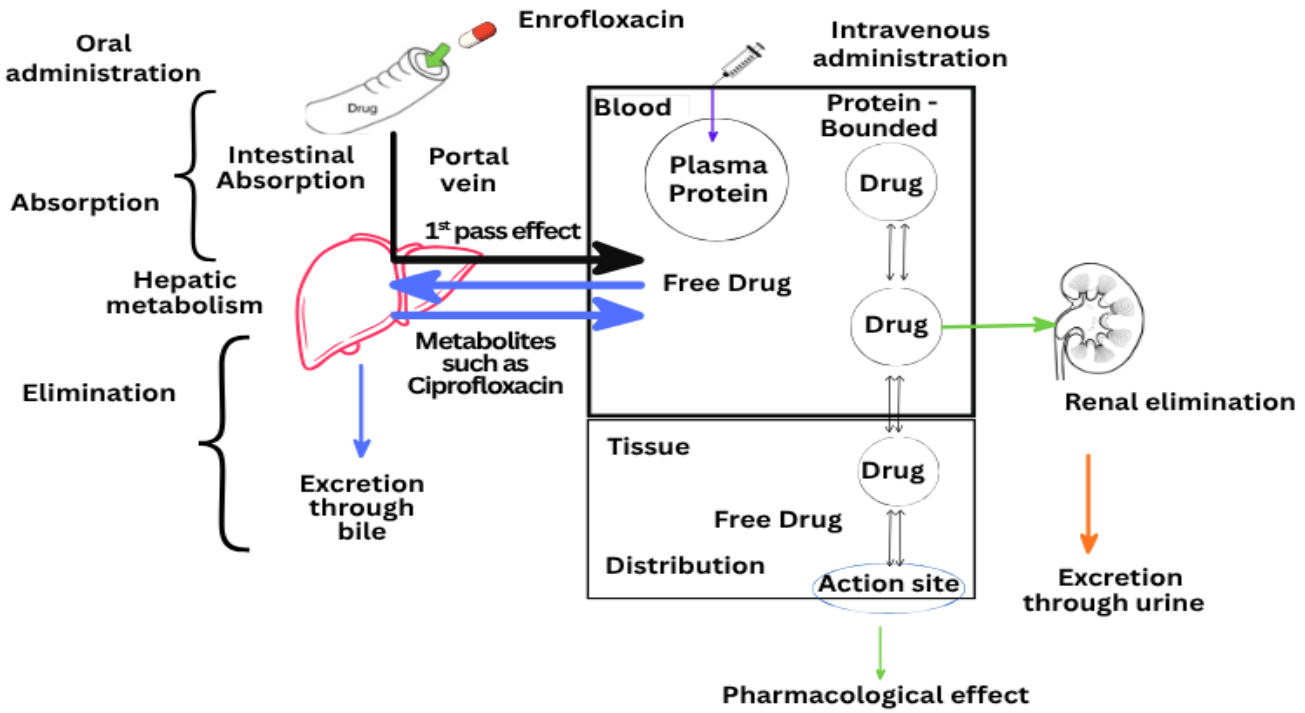

Figure 2. Pharmacokinetics involve absorption, distribution, metabolism, and elimination of drug.

Concentration-dependent Bactericidal effect

The standard enrofloxacin dosage form in chickens is 10 mg/kg per day via drinking behaviour; it can treat almost 66% of birds successfully at a time of low-level bacterial resistance, but a study reported that an optimum dose of 12.5 mg/kg/day works effectively against colibacillosis with up to 0.125 µg/mL MIC (Temmerman et al., 2021). Another study suggests that therapy of enrofloxacin-HCl boosts the bioavailability as well as absorption of enrofloxacin in broilers; however, this combination gives enhanced efficacy if a 10 mg/kg dose is administered orally via water and predicts

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high success rates near to 100% against some gram-negative bacteria such as *E. coli*, but clinical trials are still recommended to confirm it before using it in the world because real efficacy depends on the actual bacterial susceptibility in the flock (Bonassa et al., 2021). So, right administration route, right dose, bacterial susceptibility and flock health, all are these factors decide the clinical outcomes and thus targeting the optimal PK/PD parameters by right dose regimes not only cause the treatment success but also lead to delaying resistance.

Mechanism:

Enrofloxacin, an antibiotic that acts against both gram-negative and gram-positive bacteria, has two major target sites for action such as DNA topoisomerase II (gyrase) and topoisomerase IV (topo IV). However, these topoisomerase families also exist in eukaryotes, but enrofloxacin is less potent in eukaryotes as compared to the bacterial topoisomerase family (Bush et al., 2020; Dowling, 2024). GyrA and GyrB are two subunits of DNA gyrase, while ParC and ParE are two subunits of Topo IV; however, GyrA and GyrB are analogous to ParC and ParE, respectively (Grossman et al., 2023; Abdelkreem et al., 2020). GyrA and ParC carry a tyrosine residue that ensures the DNA cutting, while GyrB and ParE perform activities that are involved in the re-ligation of DNA strands (Shafiurrahman et al., 2024; Mazurek, 2024). As replication runs, supercoiling of DNA strands occurs, so gyrase binds with a DNA segment, cuts the segment in a controlled way, pushes another segment of DNA, and then rejoins the cut. This leads to negative supercoiling and alleviates tension; thus, the normal process of replication moves forward, and ATP is required during this process. It means more ATP leads to rapid activity. After replication, interlinked DNA catenanes are formed (Grabowski et al., 2022).

Topo IV can also remove some supercoiling, but its major function is to separate the catenane DNA after the replication process (Hirsch and Klostermeier, 2021). All these steps are part of the normal process of replication in bacteria, while enrofloxacin works to stop the replication process as it binds to both DNA gyrase and Topo IV (Du et al., 2023). As enrofloxacin targets both gram-positive and

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negative bacteria, when it binds with DNA gyrase, it stops the replication process in gram-negative bacteria, while in the gram-positive bacteria, the primary target site is to stop the function of Topo IV (Mahmood et al., 2024). Enrofloxacin interrupts the DNA-enzyme complex (DNA and gyrase or Topo IV) and stabilizes it, thus preventing the rejoining of the DNA strands. Resultantly, the enzyme is trapped on a segment of DNA, so gyrase suppression occurs at the upstream site of the replication fork rapidly, and in the case of Topo IV, inhibition occurs at downstream sites. At the molecular level, two molecules of enrofloxacin non-covalently bind with the tyrosine residue of the enrofloxacin–gyrase/Topo IV-DNA complex (Spencer and Panda, 2023). As the drug sticks, conformational changes occur in the enzyme; as a result, replication stops. Bacteriostatic action can occur when a low concentration of dose is administered, which may be reversible because bacteria stimulate the SOS response, stop dividing, and start to repair DNA, while a high concentration of dose can cause the bactericidal activity, leading to irreversible damage of the chromosome fragments and bacterial death (Bakhshi et al., 2024; Afzal et al., 2025). The mechanism of action of enrofloxacin is shown in Figure 3.

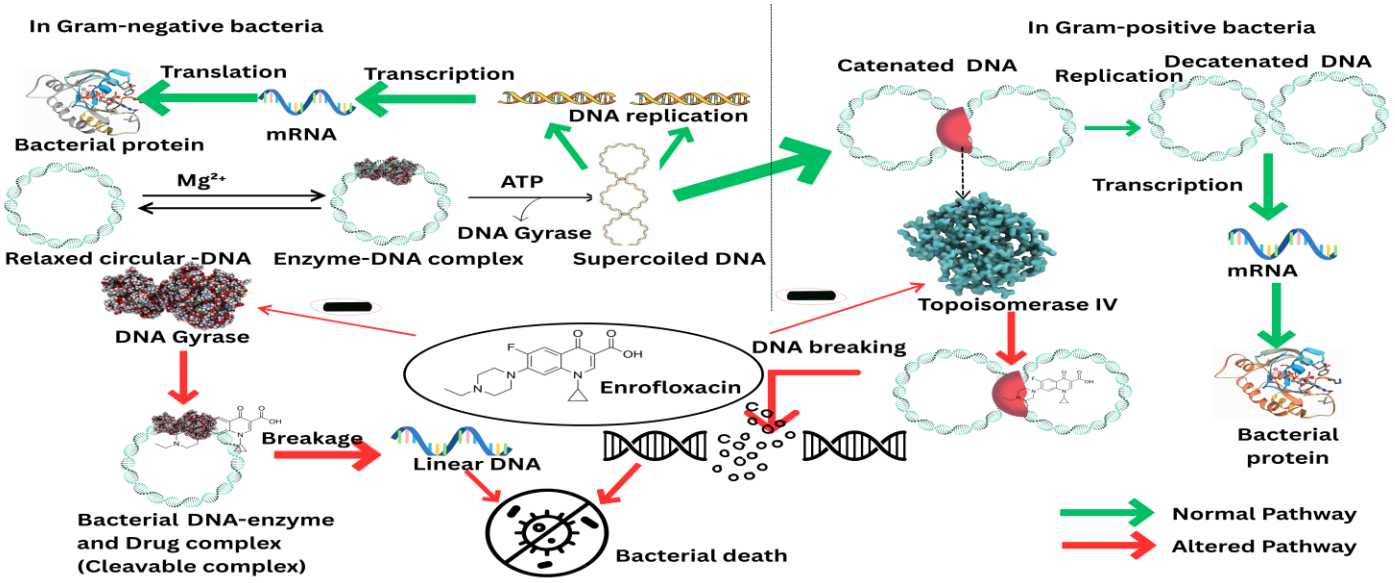

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Figure 3. Enrofloxacin performs bactericidal activity by binding with DNA gyrase and topo IV.

Mechanism of resistance

DNA gyrase and Topo IV, the major target sites of enrofloxacin, are shielded by the defensive protein that encodes the qnr gene. There are three main gene families identified, such as qnrA, qnrB, and S. Moreover, these genes are found widely in different strains of APEC because transferable plasmids in bacteria carry these genes, so one bacterial plasmid transfers resistant genes to another bacterium (Shafiurrahman et al., 2024; Zhao et al., 2022; Grabowski et al., 2022). The subunit A genes of DNA gyrase and the parC subunit genes of Topo IV carry point mutations, but mutations are less commonly identified in the gyrB and parE. Thus, the amino acid sequence alters due to point mutations. Moreover, this altered sequence of amino acids leads to a reduction in the enrofloxacin binding to the crucial enzymes. Regardless of enrofloxacin, enzymes can continue the normal process of replication and can make bacterial survival possible. The most seen mutations of amino acids in the gyrA subunit are at the S83L/D87N positions, while S80I/E84G and S80I/E84A are positioned in the case of the parC subunit; thus, these mutations cause a high level of resistance against enrofloxacin (Yoon et al., 2020).

An aac(6')-Ib-cr Gene also causes resistance to enrofloxacin, however, this gene has a special enzyme named aminoglycoside acetyltransferase that can add an acetylate, an acyl group to certain quinolones such as enrofloxacin. This modification by aminoglycoside acetyltransferase can chemically change the drug, so its binding ability to targeted enzymes is reduced (Joel et al., 2024). Another method of resistance is efflux pumps that have special genes. These genes have special proteins that form efflux pumps, which are tiny pumps located in the cell membrane of bacteria. As enrofloxacin enters the cell membrane, these pumps split out the drug from the inside to the outside environment; thus, the inside concentration of the drug reduces, so the drug does not reach the targeted enzymes (Huang et al., 2022).

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Enrofloxacin challenges and alternative approaches

For a considerable time, enrofloxacin was perceived as a potent drug used as both a prophylactic and a therapeutic agent to control colibacillosis due to its favourable penetration in tissues and fast bactericidal action (Ahmad et al., 2020). However, over time, reliance on this drug has been increasing and has led to several challenges; moreover, repeated and subtherapeutic use of enrofloxacin over time has exerted the selective pressure that has driven the emergence of resistance in APEC strains (Trinchera et al., 2025). The prevalence of enrofloxacin-resistant APEC strains and elevated levels of MICs reported by multiple studies are primarily linked with mutations in genes of DNA gyrase and Topo IV (Abdelwahab et al., 2022). Resultantly, therapeutic effectiveness declines, and treatment failures are reported increasingly, thus limiting the potential of enrofloxacin as a therapeutic agent (Gigante et al., 2024).

Apart from the accelerating resistance issue, the drug residue is also an additional concern that restricts long-term use as well as its primary therapeutic potency. Enrofloxacin and its major metabolite can accumulate in edible parts of the chicken, which may cause health problems in humans and play a role in resistance against antibiotics used in humans. The withdrawal period for ciprofloxacin metabolite and enrofloxacin may vary in chickens in different countries (Badawy et al., 2021). As claimed by the European Union Maximum Residue Limits (EU MRLs), the withdrawal period of enrofloxacin and its primary metabolite is 5 days in chicken, while Japan's MRLs claim that it is 9 days (Veerapandian and Sarathchandra, 2021). Concentration of drug residues in meat and eggs of poultry raises extreme food safety as well as public health concerns (Mesfin et al., 2024). However, in many countries, regulatory bodies are enforcing stricter controls on antibiotic use in food-producing birds as well as animals and monitoring the withdrawal intervals, emphasizing the responsible use of antimicrobials and the prohibition of sub-therapeutic practices (Afzal et al., 2025). Currently, demand for antibiotic-free poultry has been accelerated by consumers, which is compelling the poultry industry to search for sustainable alternative disease control interventions (Mateen et al., 2025). Another challenge is the administration of enrofloxacin via a drinking model

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because the consumption rate of water, which can vary with humidity, bird health, and temperature, results in deciding how much dose the bird receives via the drinking model; however, this variation can cause a sub-therapeutic level, which further leads to AMR (Zhou et al., 2021). Given these challenges, exclusive reliance on enrofloxacin alone is not a viable approach; moreover, innovative approaches are required that can reduce the APEC infection, strengthen the host immunity, and target the APEC strains through specific mechanisms. These alternative strategies may include nanoparticles, phytochemicals, essential oils, synbiotic combinations, probiotics, and phage therapy (Ike et al., 2025; Hussain et al., 2024; Maqbool et al., 2026). The administered routes, mechanisms, and outcomes of these alternatives are shown in Table 1. (Various non-antibiotic control strategies and their mechanism to reduce APEC induced colibacillosis)

Table 1. Various non-antibiotic control strategies and their mechanism to reduce APEC induced colibacillosis

Therapeutic Category	Bioactive Agent	Administered routes	Host	Study description	Mechanism	Key outcomes	References
Nanoparticles	Silver nanoparticles (Ag NPs) and zinc oxide quantum dots (ZnO QDs)	Orally administered in drinking water	Broiler chicks	Cardiac and pulmonary lesions in APEC-diseased chicks	Anti-inflammatory action and anti-microbial role of nanoparticles	Nanoparticles lessen the heart and lungs lesions.	(Afifi et al., 2025)
	Andrographi	Orally, dry	Broilers	Hematobio	Enzyme	Enhanced	(Kamil et

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Phytochemicals	*s paniculata*	leaf powder in feed		chemical parameters in infected broilers	induction, immune modulation, and antioxidant activation	lymphocyte count, albumin, PCV and Hb count. Reduced absolute heterophil count, and boost total protein in serum, Comparable levels of AST and ALT indicate normal health.	al., 2023)
	Echinacea purpurea	Orally, in feed	Broilers	Immunological, biochemical, and growth rate, and pathogenic	Immune modulation and antimicrobial activity	Improved immune system, growth performance, and reduced	

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				ity in organs infected with APEC		expression of virulence factors.	
Essential oils	Essential oils (cinnamon, clove, and lavender) interact with enrofloxacin	Orally with drinking water or feed supplementation, The topical route is less common.	Broiler-derived strains	Impact on multi drug resistant *E. coli* isolates	Boost efficacy by disrupting the cell membrane, thereby inhibiting DNA replication.	Synergistic suppression of multidrug-resistant APEC	(Zych et al., 2024)
Probiotics	Bacillus spp. (B. cereus var. toyoi, B. licheniformis, B. subtilis)	Oral (feed)	Laying hens	Mortality and growth rate, and egg quality in APEC-challenged hens	Immune modulation, changing the microbiota composition, and competitive exclusion	Promotes growth rate, egg quality, and retards the APEC growth.	(Waliaula et al., 2024)
Synbiotic combination	Enrofloxacin plus Synbiotics (probiotics	Orally in feed or water	Broiler chickens	Effect of Enrofloxacin plus Synbiotics	Enrofloxacin destabilizes the microbiome,	Synbiotics alleviate the resistome	(Temmerman et al., 2022)

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	and prebiotics)			on broiler gut resistome	while synbiotics + enrofloxacin cause stabilization of the microbiome by suppressing the antimicrobial resistance genes.	enrichment in the gut as compared to the single effect of enrofloxacin.	
Phage therapy	Escherichia phage vB_EcoS_PJ16.	Orally in chicks	Broiler chicks	Phage is isolated from wastewater of poultry farms, phage characterization, host range, genomic testing and analysis, in	Phage lytic activity. Lysis of bacterial cells, thus alleviation of the infection	Phage performed lytic activity against APEC strains; in vivo mortality decreased in the therapeutic group by	(Jhandai et al., 2024)

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				vitro evaluation, and in vivo testing on chicks for therapeutic and prophylactic analysis by oral administration.		20% and in the prophylactic group by 30%; in vitro, there was a significant reduction in APEC.	
	Escherichia phage AG-MK-2022 (Basu) and Escherichia phage VaT-2019a PE17	In vitro study, so no animal is used	Broiler chicken	Lytic phages were tested against MDR APEC strains in vitro, and their host-range and stability were checked.	Phages action is shown via bacteriolytic, or lytic cycle	Both phages killed almost 95% of APEC strains.	(Karami et al., 2024)

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Rutin intervening with quorum sensing (QS)	Rutin	In vitro study, not a study on live chicken	Chicken	In vitro, using APEC-O78, measured autoinducer-2 (AI-2) secretion, formation of biofilm, and virulence gene, and tested damage to chicken lung cells.	Inhibit AI-2 quorum sensing signaling, decreases virulence genes and lung cell damage	Rutin decreased AI-2 secretion, expression of virulence genes, biofilm formation, adhesion and lung cell damage.	(Peng et al., 2018)
Quorum sensing inhibitor (QSI) + growth inhibitor (GI)	Quorum sensing inhibitor-5 (QSI-5) + growth inhibitor-7 (GI-7) and sulfadimethoxine	Orally administered through drinking water	Broiler chicken	Chicken infected with APEC-O78, and both QSI-5 and GI-7 given as treatment	QSI-5 inhibits quorum sensing while GI-7 performs its function by inhibiting the growth of the APEC strain.	QSI-5 and GI-7 decreased mortality up to 90% and 80%, respectively. Combinat	(Helmy et al., 2023)

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combination therapy	(SDM)			separately and with combination therapy, compared with antibiotic SDM treatment, results seen over time		ion therapy also reduced the death rate by to 80%, while SDM reduced mortality by almost 70%.	
Restoring butyrate level leads to reducing APEC infection	Butyrate	Orally in drinking water	laying hens	APEC causes infection in laying hens, given antibiotic florfenicol treatment, leading to gut dysbiosis, decreasing butyrate	Gut barrier maintained by the butyrate, enhances immunity, counters dysbiosis, and restores intestinal homeostasis.	Butyrate level restored, also bacterial count decreased in spleen, improved immune systems	(Yu et al., 2025)

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				levels, and then treated with butyrate.			
Feed and water hygiene and storage	Water cleaning systems, disinfectants, mycotoxin control, and storage of feed	Sanitizers or acidifiers are applied through waterlines, and mycotoxin binders through feed.	Chickens, turkeys	Contaminated water and feed act as vectors for major bacterial and viral pathogens such as APEC and avian influenza virus in chickens and turkeys, and they also stress the	Hygiene prevents biofilm formation, decreases pathogen entry and colonization in birds' guts, and thus the pathogen transmission cycle breaks.	Enhanced water and feed hygiene that minimizes pathogen exposure and other secondary infections; hygiene reduces the need for antibiotic treatment.	(Kovács et al., 2025)

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				biosecurity implications.			
Vaccination	*Salmonella Typhimurium* (S. Typhimurium) live-attenuated vaccine indicating virulence genes of APEC such as P fimbriae and iutA and some associated antigens.	Oral, intramuscular, or subcutaneous routes depending on protocol.	Broiler chickens	To measure immunity response and survival, chickens were given primary and then booster shots, then live APEC was put into the air sacs of chickens, and cytokines and antibody titers were also measured.	Oral Salmonella delivers APEC antigen to gut mucosa; mucosal immunity and systemic immunity are induced, and cytokine responses play a role in protecting against strains of APEC.	80% protection was seen in the vaccinated + booster chickens' group, which also observed high levels of antibodies like IgG/IgA and cytokines such as IFN-γ, IL-2, and IL-6.	(Chagas et al., 2024)

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Future perspectives and challenges

The current dependence on enrofloxacin and other alternative strategies against APEC-induced colibacillosis is satisfactory up to a certain extent; however, rapid development of resistance makes its use unsustainable. Moving ahead, precision-guided, sustainable, smart, and long-lasting strategies should be adopted to control colibacillosis. A single magic dose will not define the future against APEC control, but strategic incorporation of several innovative and synergistic approaches should be adopted. Disease-resistant broilers can carry such genes in their gut microbiome that may produce antimicrobial peptides (AMPs). Metagenomic mining is involved in future research; after gene sequencing of the gut flora of disease-resistant chickens, the specific genes can be isolated and then cloned into safe probiotics, such as yeast, and inserted into sick chickens, leading to the production of APMs, which can act as therapeutic agents against APEC.

In the future, instead of traditional methods, the targeted sonication method will be used, and experimentation should be conducted on it. Targeted sonication will break the APEC biofilm mechanically; thus, bacteria will be killed by the host immune system instead of conventional antibiotic medication. Smart nanoparticles will also be used in the future to detect a specific strain of APEC when these nanoparticles are equipped with special molecules, such as antibodies, that may precisely find and stick to a particular APEC strain by a specific surface protein. After attachment, a payload system is activated that will release the antibiotics in tiny amounts to kill the targeted APEC strain by using magnetic and light energy. This targeted treatment ensures that medicine is transported only to the focus site, which can also reduce the risk of resistance. Work should be done in the future on synergistic combinations of nanoparticles with probiotics and phytochemicals that should target the specific strain of APEC to get more effective and accurate results to control the disease, and exploring new phytochemicals will help to make a residue-free and safe strategy to control APEC strains that can decrease the conventional antibiotic use in poultry (Zahid et al., 2025; Fatima et al., 2026).

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Conclusion

APEC-induced colibacillosis can be treated by enrofloxacin, and it has been used as a therapeutic agent, its PK/PD model helps in understanding the drug efficacy and performance but its concentration dependent dose makes its efficacy much greater but this thing leads to misuse and due to its misuse and multiple mechanisms such as QRDR and PMQR involving mutations in the genes of bacterial enzymes, it accelerates the resistance, causing it to compromise its clinical effectiveness, but some sustainable strategies such as non-antibiotics strategies as an alternative can be used that can reduce the effect of APEC strains. Future perspectives stress that long-lasting and precision-guided methods, such as metagenomic mining, novel phytochemicals, and smart nanoparticles, as well as the targeted sonication method, should be adopted to minimize the spread and to control colibacillosis.

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