Research Article

Evaluation of the Therapeutic Efficacy of Pefloxacin and Florfenicol Combination in Broilers Experimentally Challenged by *Escherichia coli*

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ABSTRACT

Modification of the curative efficiency of one or both of the administered drugs and minimized the drug resistance to take place through antimicrobial combination. In this study, the potential interaction effects of pefloxacin and florfenicol and their efficiencies were inspected on experimentally challenged broilers by *Escherichia coli* (*E. coli*) at 23 days of age. Challenged broilers were treated either by pefloxacin (10 mg kg^{-1}) or florfenicol (30 mg kg^{-1}) orally, once per day for 5 successive days. Their combinations of either the recommended or half-therapeutic doses. Blood sampling (n=5) was collected from all groups at 2nd, 4th and 5th days during the treatment course and at 7th and 14th days post treatment. The obtained results revealed that pefloxacin and florfenicol combination minimized the severity of clinical signs due to *E. coli* infection. The drug combination of the recommended dose induced a significant ($P<0.05$) changes in blood picture and liver biochemical parameters. Side effects of chemotherapeutic agents were restricted and generality of the indices returned to normal level post two weeks after cessation of both drug administrations.

Keywords: Therapeutic, challenge, antimicrobial drug combination, Broilers; *E. coli.*

INTRODUCTION

E. coli still stands to be a global serious issue causing mortalities and requisitions leading to a significant loss in chicken production[1,2].

Therefore, there is a real need to find new strategies of antimicrobial usage for the controlling of colibacillosis in poultry production[3]. Antimicrobials therapy of broiler flocks exposed to colibacillosis has met with much less success recently than in the preceding due to expanded antimicrobials impedance and the scarcity of unprecedented antimicrobials to take their place. A very efficacious antimicrobials, pefloxacin and florfenicol, were participated to a large degree as prophylactic medicament and as of therapeutic values as well [3, 4].

Florfenicol is a wide range bacteriostatic antibiotic, against different types of gram-negative and gram-positive bacteria with special attention to *Escherichia coli*[5, 6]. In addition, florfenicol is highly dynamic at lower concentrations, rather than thiamphenicol and chloramphenicol, against its constitutional analogs-resistant bacterial pathogens. For this reasons, florfenicol is confirmed in the European countries for use in poultry production [7, 8].

Pefloxacin is a made-up fluoroquinolone antibacterial, prime spectrum remedy. It has an outstanding antibacterial efficacy against most gram-negative and gram-positive bacteria[9]. Pefloxacin is a bactericidal compound, this action results from interference with the activity of DNA gyrase and topoisomerase IV, which are important for the transcription and replication of bacterial DNA[10, 11]. Pefloxacin shows a good absorption with both high bioavailability and excellent tissue penetration[12]. The extended half-life, like other fluoroquinolones is referred to pefloxacin is metabolized in the liver and gave rise to norfloxacin, which is itself a bactericidal compound and licensed for use in veterinary medicine[13, 14].

Because of co-administration of different antimicrobials may predominatingly result in an unpredictable therapeutic result as to minimize the therapeutic efficacy or increased the toxicity among the administered drugs through various pharmacokinetic interactions[15, 16].

Unfortunately, the literatures concerning use of pefloxacin and florfenicol to treat *E. coli* or other infections in poultry are scarce. These data are required for determining dosage schedules for clinical use in birds. Therefore, this study has been designed to investigate the efficacy of pefloxacin and florfenicol combination usage in *E. coli* infected chicken on some hematological and biochemical parameters that indicating the general health condition in chicken.

MATERIALS AND METHODS

Birds

Unvaccinated two hundred and forty, one day old broiler chicks (Hubbard x Hubbard) of mixed sexes were used in the study. The chickens were obtained from a commercial hatchery. They were placed in the animal house at Faculty of Veterinary Medicine, Damanhour University. The birds were monitored for two weeks for any apparent clinical signs of disease prior to drug(s) administration. The temperature was maintained at 25±2°C and humidity at 45–65%. The chickens had free access to water and food without additives, such as antibiotics and growth

promoters. Use of animals in this study was in accordance with Good Laboratory Practice Standards.

Chemotherapeutic agents

Peflodad® (Pefloxacin 10%) was obtained from Dar Aldawa Veterinary and Agriculture Industrial Co. Ltd. Jordan. Each mL contains 100 mg of pefloxacin base. The recommended dose was 10 mg kg^{-1} for five successive days in the drinking water [17].

Floricol® (Florfenicol 10%) was obtained from Pharma Swede Co., Egypt. Each mL contains 100 mg of florfenicol base. The recommended dose was 30 mg kg^{-1} for five successive days in the drinking water [18].

E. coli challenge

E. coli strain O78 was obtained from the Animal Health Research Institute, Dokki, Giza, Egypt. Broiler chickens of the treated groups were orally challenged with 1ml of inoculums containing 10^9 CFU (colony forming unit) at 23 days of age as mentioned before [19].

Efficacy of pefloxacin or florfenicol against *E. coli*

At first, the sensitivity of the used *E. coli* strain to pefloxacin or florfenicol, the antibiotic sensitivity test was done using disc diffusion method. Enrofloxacin, norfloxacin, ciprofloxacin and chloramphenicol discs (Oxoid, UK) were used to compare their zones of inhibition with pefloxacin or florfenicol. The diameters of inhibition zones were interpreted by referring to the tables [20].

Experimental Design

The chickens were allocated into six equal groups (each of 40):

Group 1. Birds were served as a control group (non infected – non treated).

Group 2. Birds were served as infected – non treated group.

Group 3. Birds were challenged with *E. coli* and treated orally with pefloxacin (10 mg kg^{-1}), after starting of symptoms and available for 12 hours, once daily for five successive days.

Group 4. Birds were challenged with *E. coli* and treated orally with florfenicol (30 mg kg^{-1}) after starting of symptoms and available for 12 hours, once daily for five successive days.

Group 5. Birds were challenged with *E. coli* and treated orally with a combination of pefloxacin (5 mg kg^{-1}) available for 12 hours then florfenicol (15 mg kg^{-1}) available for the next 12 hours daily for five successive days, starting after starting of symptoms.

Group 6. Birds were challenged with *E. coli* and treated orally with a combination of pefloxacin (10 mg kg^{-1}) available for 12 hours then florfenicol (30 mg kg^{-1}) available for the next 12 hours for five successive days, after starting of symptoms.

The clinical signs of *E. coli* infection in broilers were diarrhea, lack of appetite and ruffled feathers. Before starting the treatment, three infected birds were slaughtered and examined for post mortem lesions, and for bacterial isolation from liver and heart. At necropsy of the birds liver, air sac and heart were aseptically excised and swabs from liver and heart were incubated in beef infusion broth and then plated on MacConkey agar at 37°C for 24 hours. The serogroup of *E. coli* were confirmed by agglutination reaction with *E. coli* O78 antiserum.

Clinical symptoms and mortality rate were monitored along the experimental period. Two blood samples were taken on 2nd, 4th and 5th days during the treatment and on the 7th and 14th days post treatment for hematological and biochemical analysis.

Hematological Analysis

Total red blood cell (TRBC) and total white blood cell count (TWBC) were determined by a manual method using hemacytometer[21]. Packed cell volume (PCV) was measured by a standard manual technique using microhematocrit capillary tubes. Hemoglobin concentration (Hb) was measured by cyanmethemoglobin method[22].

Biochemical Analysis

Serum biochemical parameters like alanine transaminase (ALT, EC 2.6.1.2) and aspartate transaminase (AST, EC 2.6.1.1), total proteins, albumin, bilirubin, uric acid and creatinine were analyzed by commercially available kit methods. Globulins were estimated by electrophoretic analysis of serum protein.

Statistical analysis

The descriptive data were presented as the Mean ± SE. The statistical differences were calculated based on two way test of ANOVA and $p<0.05$ is considered as significant between the groups. All statistical analyses were carried out using SigmaStat for Windows, version 2.0, Jandel Corp., San Rafael, CA, U.S.A.

RESULTS AND DISCUSSION

Colibacillosis points to systemic infection caused by avian pathogenic *Escherichia coli* [23]. It is a globally dispersal infectious disease that represents a main concern in the poultry farming. Infection of broiler chickens with *E. coli* usually takes place at 2-8 weeks of age with colisepticemia and respiratory problems, reduced feed intake, growth reduction with high death rates[24,25].

Functional monitoring extents against *E. coli* infection are not obtainable at the moment. Consequently, the general demand is the synergistic effect of antimicrobials to minimize the possibility of mutations occurred to genes present on the bacterial plasmids usually encode resistance to these antimicrobials and transferring resistance from generation to another [19].

Inoculation with *E. coli* O78 induced severe colibacillosis in chickens characterized by depression, diarrhea, lack of appetite, ruffled feathers and some respiratory manifestations.

The results of *in vitro* antibiotic sensitivity test showed that the used *E. coli* challenge strain (O78) was sensitive to pefloxacin and florfenicol than the other antibacterial chemotherapeutic discs (enrofloxacin, norfloxacin, ciprofloxacin and chloramphenicol).

Previously co-administration of pefloxacin (3rd generation of fluoroquinolones) together with florfenicol may overcome the problem of drug shortage in avian colibacillosis treatment [26].

Mortality rate percentage

The mortality rate in each group was recorded throughout the experimental period in Table 1. Infection with *E. coli* induced (20%) mortalities in chickens and this percent was reduced to (12.5%) due to pefloxacin or florfenicol treatments. While drug combinations in different doses decreased the mortality rate to (5%).

Challenged birds with *E. coli* showed a cumulative mortality rate (20%) which were higher than birds in pefloxacin, florfenicol and pefloxacin/florfenicol (therapeutic doses) treated group (12.5, 12.5 and 5%, respectively). As *E. coli* (O78) pathogenicity can induce high rate of morbidity and mortalities within a short time among susceptible birds[27]. Also, the finding of this work is in agreement with those published before[19, 28]. Who reported that sarafloxacin treatment of broiler chickens could reduced mortalities from 75% in *E. coli* infected birds to 27% in infected medicated ones. Although *E. coli* multiplied in the respiratory tract was significantly reduced by both enrofloxacin treatment and the florfenicol treatment, but with the enrofloxacin treatment showing significantly better penetration power to respiratory tract and so the reductions of *E. coli* counts more than the florfenicol treatment[29].

The mechanism of virulence of *E. coli* strains pathogenicity to birds is attributed to the prevalence of toxic factors produced by some *E. coli* strains isolated from visceral organs of chickens with colisepticemia and from feces of healthy chickens were capable of production of different types of enterotoxins (verotoxins, cytotoxic necrotizing factors, enterohemolysin and other types of colicins with necrotic and lethal properties[30]). Moreover, these findings explained also the decreasing of erythrocytic indices, hepatic and kidney function parameters.

Post mortem examination percentage

Table 2 recorded the lesion scores in each group throughout the experimental period. Perihepatitis and pericarditis were the major post mortem lesions mentioned in challenging non-treated birds (92.59 and 88.88%, respectively). These ratios were sharply dropped from florfenicol treatment **(**11.11 and 18.52%, respectively**)**, better than pefloxacin treatment **(**11.11 and 22.22%, respectively**)**. Treatment of *E. coli* challenged chickens with either a combination of half the therapeutic doses of pefloxacin and florfenicol or a combination of the therapeutic doses, minimized airsacculitis percent by (22.22%) in comparison with pefloxacin or florfenicol alone (40.74%).

Table 1: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and / or florfenicol (30 mg kg^{-1}) for five successive days on the mortality rate percentage of experimentally infected chickens with *E. coli* (n=40).

Groups	Number of dead birds	%
Group 1	0	0
Group 2	8	20
Group 3	5	12.5
Group 4	5	12.5
Group 5	2	5
Group 6	2	5

In the present study, experimentally infected birds with *E. coli* (O78) induced typical colibacillosis as depression, decrease food intake, weight loss, respiratory signs and diarrhea. Moreover, *E. coli* infection produced some gross lesions as airsacculitis, pericarditis and perihepatitis. Also, infection of chickens with *E. coli* induced high mortality rate. The obtained results came close with that reported before[31-33]. They mentioned previously that *E. coli* infection in chickens caused airsacculitis, pericarditis, perihepatitis, enteritis and respiratory manifestations.

Destruction of epithelial cells have been occurred by *E. coli* because of the production of toxins, providing an increase in available nutrients due to leakage of plasma proteins that will create an environment opportune for propagation of commensally microorganisms as *C. perfringens* may lead to subsequent hemorrhagic enteritis[16, 34].

Presented data in this study clearly demonstrated that infected groups with *E. coli* (O78) showed lesions beginning with the 2nd day post challenge including septicemia and serous to fibrinous airsacculitis, pericarditis and perihepatitis either in dead or sacrificed birds. Also, these data supported by hematological and biochemical interpretations as a result of *E. coli* infection.

***E. coli* reisolation percentage**

Data of *E. coli* reisolation from internal organs of examining birds were recorded in Table 3. *E. coli* reisolation rate from liver and heart was dropped from (85.18 and 77.78 %, respectively) to (29.62 and 25.93 %, respectively) due to pefloxacin treatment, which considered more prominent than florfenicol treatment (33.33 and 29.62%, respectively).

Treatment of *E. coli* challenged chickens by a combination of the therapeutic doses of pefloxacin and florfenicol reduced *E. coli* reisolation rate from liver and heart of

(29.62 and 25.93%, respectively) with respect to those data recorded in birds treated with a combination of half the therapeutic doses of pefloxacin and florfenicol (40.74 and 33.33, respectively).

Table 2: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and / or florfenicol (30 mg kg^{-1}) for five successive days on gross pathological lesions percentage of experimentally infected chickens with *E. coli* (n=3 birds per day, total 27 birds).

Groups	lesion	Number of positive cases										
		1st	2nd	3rd	4th	5th	6th	7th	14th	21st	Sum.	%
Group 1	perihepatitis	0	0	0	0	0	0	0	0	0	0	0
	pericarditis	0	0	0	0	0	0	0	0	0	0	0
	airsacculitis	0	0	0	0	0	0	0	0	0	0	0
Group 2	perihepatitis	0	0	0	3	3	4	5	5	5	25	92.59
	pericarditis	0	2	2	4	4	3	3	3	3	24	88.88
	airsacculitis	2	2	2	2	3	3	3	3	3	23	85.18
Group 3	perihepatitis	0	0	1	1	1	0	0	0	0	3	11.11
	pericarditis	0	2	1	1	1	1	0	0	0	6	22.22
	airsacculitis	2	2	2	2	1	1	1	0	0	11	40.74
Group 4	perihepatitis	0	0	1	1	1	0	0	0	0	3	11.11
	pericarditis	0	1	1	1	1	1	0	0	0	5	18.52
	airsacculitis	2	2	2	2	2	1	0	0	0	11	40.74
Group 5	perihepatitis	0	0	1	1	1	0	0	0	0	3	11.11
	pericarditis	0	2	2	1	1	1	1	1	0	9	33.33
	airsacculitis	1	1	1	1	1	1	0	0	0	6	22.22
Group 6	perihepatitis	0	0	1	1	0	0	0	0	0	2	7.41
	pericarditis	0	1	1	1	1	0	0	0	0	4	14.81
	airsacculitis	1	1	1	1	1	1	0	0	0	6	22.22

Table 3: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and / or florfenicol (30 mg kg^{-1}) for five successive days on *E. coli* reisolation percentage from internal organs of experimentally infected chickens with *E. coli* (n=3 birds per day, total 27 birds).

Groups	lesion	Number of positive cases										
		1st	2nd	3rd	4th	5th	6th	7th	14th	21st	Sum.	%
Group 1	liver	0	0	0	0	0	0	0	0	0	0	0
	heart	0	0	0	0	0	0	0	0	0	0	0
Group 2	liver	2	2	2	3	3	2	3	3	3	23	85.18
	heart	2	2	2	3	3	3	2	2	2	21	77.78
Group 3	liver	0	0	2	2	2	2	0	0	0	8	29.62
	heart	1	1	1	1	1	1	1	0	0	7	25.93
Group 4	liver	2	2	2	1	1	1	0	0	0	9	33.33
	heart	2	2	1	1	1	1	0	0	0	8	29.62
Group 5	liver	2	2	2	2	2	1	0	0	0	11	40.74
	heart	1	1	2	1	1	1	1	1	0	9	33.33
Group 6	liver	2	2	2	1	1	0	0	0	0	8	29.62
	heart	2	2	1	1	1	0	0	0	0	7	25.93

Infected groups with *E. coli* (O78) showed lesions at the 1st day post challenge that either include after septicemic reaction and fibrinous airsacculitis, pericarditis and perihepatitis in dead or sacrificed birds. Administration of pefloxacin, florfenicol and pefloxacin/florfenicol (therapeutic doses) reduced the macroscopic lesion score in the medicated birds than non-medicated infected ones. The lesions were completely absent approximately week after pefloxacin, florfenicol and pefloxacin/florfenicol (therapeutic doses) medication.

The necropsy findings of this experiment are supported by previous reports[35, 36]. Who observed lesions of fibrinous airsacculitis, pericarditis and perihepatitis after systemic inoculation of *E. coli* (O78) in chickens.

Regarding the results of mortalities, organ lesion scores and isolation of the organism that are used as criteria for evaluation of *E. coli* infection in birds in this work. The count of *E. coli* isolated from the sinuses was significantly reduced only by enrofloxacin treatment, appeared to be more effective than florfenicol treated group. For the

liver and heart, no positive samples were encountered, so significant differences between the treatments did not occur. Fortunately, these published data agree with our findings of bacterial reisolation from internal organs of experimentally infected chickens with *E. coli* [29].

Histopathological observations detected latterly[32, 37]. Who observed that *E. coli* (O78) induced perihepatitis, degenerative changes in hepatocytes as well as leukocytes infiltration and dilatation of the portal blood vessels. In addition, they found severe pericarditis and myocardial leukocytic infiltration. These observations came in the same line with an elevated leukocyte count, and confirmed abnormal hepatic function parameters recorded in our study.

Improving the health condition of the birds caused by pefloxacin treatment may be related to several faces such its bactericidal broad spectrum effect as a result of inhibiting the structure and function of DNA gyrase and reflected *in vitro* pefloxacin antibiogram good results.[38-40] Together with pefloxacin properties (rapid absorption, outspread distribution, swift elimination and effective sustenance of plasma concentrations) facing bacterial infections as mentioned before.

Moreover, it has been reported that florfenicol showed greater activity than chloramphenicol and thiamphenicol, especially against *E. coli*. Florfenicol has topmost pharmacological and pharmacokinetics features over some other antimicrobials used in the poultry industry. This drug is described by high bioavailability (F>80%), perfect tissue penetration and rapid elimination, which are substantial for the systemic treatment in poultry industry [8].

Hematological findings

Treatment of *E. coli* challenged chickens by a combination of the therapeutic doses of pefloxacin and florfenicol for two days**,** significantly ($p<0.05$) increased the erythrocytic count (10^6 mm^{-1}), hemoglobin concentration (%) and packed cell volume percent (2.34±0.13, 7.85±0.12 and 28.88±3.34, respectively). These results continued in a progress manner until 14th days post treatment (2.69±0.11, 8.82±0.14 and 33.64±2.42, respectively) in comparison with a combination of half the therapeutic doses of pefloxacin and florfenicol treatment (Table 4).

Total leucocytic count (×10^3 μl^{-1}) remains significantly elevated in *E. coli* challenged chickens treated by a combination of half the therapeutic doses of pefloxacin and florfenicol till the 14th day post treatment (23.28±1.27) in comparison with pefloxacin, florfenicol and their combination in the therapeutic doses (20.82±3.21, 20.64±1.61 and 20.32±2.21, respectively) Table 5.

Total erythrocytic count, hemoglobin concentration, packed cell volume and total leucocytic count, after oral administration of pefloxacin (10 mg kg^{-1}) and /or florfenicol (30 mg kg^{-1}) for five successive days, are improved after the 2nd day after treatment.

The hematological results mentioned in this study are coming close to that reported previously[41]. They mentioned that enteritis could deteriorate erythrocyte indices.

The maximum plasma drug concentration and time to reach maximum plasma drug concentration of pefloxacin were 3.78 microg ml^{-1}, and 3.33 hour, respectively after a single oral administration of pefloxacin (10 mg kg^{-1}) in chickens [14].

In addition, fluoroquinolones subsequently, have the chance to affect their own half-lives through inhibition of the metabolizing oxidases enzyme system should always be considered when they are used in animals, especially when given in combination with other drugs [42].

Fluoroquinolones have their relative safety character among other antibacterial agents, by their lower minimum inhibitory concentrations (MIC), which may reach to 2.0 ug mL^{-1}, their wide spectrum activity, and their character of leaving low permissible limit of residue in edible tissues which promoted their use as drug of choice in veterinary practice [42].

Florfenicol (30 mg kg^{-1}) was absorbed rapidly after oral administration to *E. coli* infected broiler chickens and plasma concentration of florfenicol, maximum plasma concentrations (C_{max}) was 3.82 µg mL^{-1} at approximately one hour with the oral bioavailability (%) ranged from 63 to nearly 71[43, 44] .

Florfenicol efficacy is closely correlated with concentrations maintained above the MIC for a longer proportion of the interdosing interval (T>MIC). The minimum inhibitory concentration of florfenicol for *E. coli* is 4 µg mL^{-1} and florfenicol should be given at 30 mg kg^{-1} twice per day to maintain its therapeutic concentration in plasma over 24 hour to treat colibacillosis [45]. In a previous study, it is likely that florfenicol will need to be given at a dosage of > 20 mg kg^{-1} for 24 hour to maintain therapeutic concentrations or continued in water 3-5 days to treat colibacillosis[46].

Serum biochemical findings

Both ALT (IU L^{-1}) and AST (IU L^{-1}) values remain significantly ($p<0.05$) elevated from the 2nd day during treatment until the 14th day post treatment (90.12±3.64 and 32.40±2.33; 84.75±3.12 and 25.45±1.35, respectively) as a result of treatment by a combination of half the therapeutic doses of pefloxacin and florfenicol in comparison with pefloxacin and florfenicol combination of their therapeutic doses (84.85±6.54 and 21.34±3.56; 70.32±5.42 and 15.86±4.22, respectively) Table 6.

Serum total bilirubin (mg dl^{-1}) values significantly ($p<0.05$) elevated from the 4th till the 5th day of treatment in both florfenicol and pefloxacin/florfenicol combination (therapeutic doses) treated groups (0.41±0.01 and 0.41±0.01; 0.41±0.01 and 0.43±0.01, respectively), and then declined to normal levels when compared with other groups (Table 6).

Table 4: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and /or florfenicol (30 mg kg^{-1}) for five successive days on total erythrocytic count, hemoglobin concentration and packed cell volume values of experimentally infected chickens with *E. coli* (n=5).

Parameters	Erythrocytic count (10^6 mm^{-1})					Hemoglobin concentration (%)					Packed cell volume (%)				
	During treatment			Post treatment		During treatment			Post treatment		During treatment			Post treatment	
Groups	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day
Group 1	2.77 ± 0.11^{a}	2.70 ± 0.10^{a}	2.70 ± 0.10^{a}	2.71± 0.12^{a}	2.72 ± 0.04^{a}	9.82 ± 0.18^{a}	9.85 ± 0.15^{a}	9.80 ± 0.22^{a}	9.83 ± 0.12^{a}	9.86 ± 0.12^{a}	32.51± 1.12^{a}	33.51± 2.42^{a}	31.51± 1.32^{a}	33.51± 1.42^{a}	34.51± 1.45^{a}
Group 2	2.33 ± 0.05^{b}	2.26 ± 0.07^{c}	2.22 ± 0.11^{c}	2.24 ± 0.10^{c}	2.20 ± 0.12^{c}	7.84 ± 0.11^{b}	7.81 ± 0.12^{c}	7.80 ± 0.10^{c}	7.81 ± 0.11^{c}	7.80 ± 0.14^{c}	28.55 ± 2.28^{b}	27.84 ± 1.38^{c}	27.36 ± 1.62^{c}	27.55 ± 1.34^{c}	27.28± 2.10^{c}
Group 3	2.39 ± 0.12^{b}	2.44 ± 0.11^{b}	2.52 ± 0.14^{b}	2.51 ± 0.11^{b}	2.52 ± 0.13^{b}	7.83 ± 0.10^{b}	8.45 ± 0.15^{b}	8.55 ± 0.17^{b}	8.50 ± 0.14^{b}	8.54 ± 0.10^{b}	28.73 ± 1.38^{b}	29.50± 1.12^{b}	30.15± 1.04^{b}	31.21± 1.39^{b}	32.60± 1.43^{b}
Group 4	2.38 ± 0.11^{b}	2.42 ± 0.11^{b}	2.50 ± 0.14^{b}	2.52 ± 0.11^{b}	2.52 ± 0.14^{b}	7.87 ± 0.11^{b}	8.44 ± 0.14^{b}	8.56 ± 0.13^{b}	8.57 ± 0.12^{b}	8.58 ± 0.15^{b}	28.66 ± 2.28^{b}	29.36± 2.13^{b}	31.51± 1.33^{b}	31.66± 1.12^{b}	32.51± 1.42^{b}
Group 5	2.36 ± 0.14^{b}	2.35 ± 0.11^{c}	2.34 ± 0.12^{c}	2.35 ± 0.10^{c}	2.33 ± 0.11^{c}	7.83 ± 0.10^{b}	7.44 ± 0.11^{c}	7.82± 0.12^{c}	7.80 ± 0.10^{c}	7.80 ± 0.15^{c}	28.67 ± 2.33^{b}	27.64 ± 1.97^{c}	27.49 ± 1.10^{c}	27.64 ± 1.32^{c}	27.82± 1.30^{c}
Group 6	2.34 ± 0.13^{b}	2.44 ± 0.11^{b}	2.55 ± 0.10^{b}	2.57 ± 0.11^{b}	2.69 ± 0.11^{ab}	7.85 ± 0.12^{b}	8.46 ± 0.15^{b}	8.64 ± 0.10^{b}	8.74 ± 0.16^{b}	8.82 ± 0.14^{b}	28.88 ± 3.34^{b}	30.51± 1.32^{b}	31.25± 1.54^{b}	31.41± 1.34^{b}	33.64± 2.42^{ab}

Values are expressed as Mean±SE; The means with different superscripts in the same column indicate significantly different, (p<0.05).

Table 5: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and /or florfenicol (30 mg kg^{-1}) for five successive days on the total leucocytic count of experimentally infected chickens with *E. coli* (n=5).

Parameter	Total leucocytic count (× 10^3 μl^{-1})				
	During treatment			Post treatment	
Groups	2nd day	4th day	5th day	7th day	14th day
Group 1	19.26 ± 1.41^{b}	19.41 ± 1.27^{d}	20.26 ± 1.13^{c}	20.75 ± 1.42^{c}	21.18± 1.42^{c}
Group 2	24.37 ± 1.27^{a}	25.08 ± 1.12^{a}	26.49 ± 1.22^{a}	26.59 ± 1.23^{a}	26.91± 1.23^{a}
Group 3	23.49± 1.02^{a}	21.60± 1.03^{c}	21.26± 1.23^{c}	20.90 ± 1.67^{c}	20.82 ± 3.21^{c}
Group 4	23.26± 1.12^{a}	21.76± 1.03^{c}	21.32± 1.27^{c}	20.74 ± 1.47^{c}	20.64 ± 1.61^{c}
Group 5	23.28 ± 1.95^{a}	23.59 ± 0.94^{b}	24.90 ± 1.87^{b}	24.75 ± 1.49^{b}	23.28 ± 1.27^{b}
Group 6	23.37± 1.44^{a}	21.57± 1.03^{c}	20.97 ± 1.71^{c}	20.69 ± 1.07^{c}	20.32 ± 2.21^{c}

Values are expressed as Mean±SE; The means with different superscripts in the same column indicate significantly different, (p<0.05).

Serum total proteins (g dl^{-1}), serum albumin (g dl^{-1}) remains significantly (p<0.05) reduced from the 2nd day during treatment till the 14th day post treatment (2.56±0.04 and 0.83±0.01; 3.13±0.11 and 1.12±0.05, respectively) as a result of treatment by a combination of half the therapeutic doses of pefloxacin and florfenicol in comparison with pefloxacin and florfenicol combination of their therapeutic doses (2.97±0.13 and 1.25±0.02; 4.16±0.11 and 1.89±0.04, respectively) Table 7.

On the other hand, the treatment by a combination of half the therapeutic doses of pefloxacin and florfenicol significantly (p<0.05) failed to provoke the serum globulin (g dl^{-1}) level starting from the 4th day during treatment (1.77 ± 0.07) forwarded to the 14th day post treatment (1.84±0.03) vs. treatment by pefloxacin and florfenicol combination of their therapeutic doses (1.74±0.02 and 2.12±0.02, respectively) Table 7.

Serum uric acid (mg dl^{-1}) and serum creatinine (mg dl^{-1}) values showed no significant changes among pefloxacin, florfenicol and their combination (either half and therapeutic doses) Table 8.

Following pharmacokinetic/pharmacodynamic interaction of pefloxacin and florfenicol, we could approve that half dose combination of previous drugs unable to overcome the colibacillosis in chickens and based also upon the hematological and biochemical findings recorded in this data.

Florfenicol was found to have adverse effects on the humoral immune response in healthy chicks, and the effects were dependant on the dosages of the drug administered. The mechanism of immunosuppression induced by florfenicol may be the same as that of chloramphenicol and thiamphenicol through protein inhibition[47]. This could facilitate another affection(s) that attributed such changes to kidney dysfunction as a consequence to renal tubular damage [48].

On the other hand, fluoroquinolones, deposited in the form of crystals (needle-shaped crystals), also, AST, ALT, alkaline phosphatase, and blood urea nitrogen (BUN) may be increased[9]. Previous findings could explain our recorded data about the elevation of serum creatinine nearly on the 7th day post treatment.

Table 6: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and /or florfenicol (30 mg kg^{-1}) for five successive days on serum alanine aminotransferase enzyme (ALT) , serum asparatate aminotransferase enzyme (AST) and serum bilirubin values of experimentally infected chickens with *E. coli* (n=5).

Parameters	Serum Alanine Aminotransferase (IU L^{-1})					Serum Asparatate Aminotransferase (IU L^{-1})					Serum Bilirubin (mg dl^{-1})				
	During treatment			Post treatment		During treatment			Post treatment		During treatment			Post treatment	
Groups	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day
Group 1	50.12± 3.11^{c}	53.10± 2.36^{c}	49.54 ± 2.85^{c}	50.34 ± 2.45^{c}	52.86 ± 4.12^{c}	9.85± 0.35^{c}	9.45± 0.62^{c}	8.97± 0.38^{c}	8.78± 0.52^{c}	7.89± 0.84^{c}	0.31± 0.01^{a}	0.30± 0.01^{b}	0.30 ± 0.01^{b}	0.29 ± 0.01^{a}	0.29 ± 0.01^{a}
Group 2	94.44 ± 4.53^{a}	96.46 ± 4.56^{a}	94.66 ± 4.35^{a}	93.52 ± 6.34^{a}	85.74 ± 2.96^{a}	34.15 ± 2.84^{a}	34.56 ± 2.79^{a}	33.84 ± 2.36^{a}	31.78 ± 3.54^{a}	29.52± 1.45^{a}	0.29 ± 0.01^{a}	0.28 ± 0.01^{b}	0.29 ± 0.01^{b}	0.28 ± 0.01^{a}	0.27± 0.01^{a}
Group 3	82.33 ± 4.10^{b}	79.44 ± 2.15^{b}	77.36 ± 4.55^{b}	74.47 ± 2.51^{b}	68.47 ± 2.33^{b}	18.45 ± 1.35^{b}	18.12± 1.42^{b}	17.14± 1.28^{b}	16.66± 1.39^{b}	15.42± 1.64^{b}	0.32± 0.01^{a}	0.30± 0.01^{b}	0.32 ± 0.01^{b}	0.27± 0.01^{a}	0.31 ± 0.01^{a}
Group 4	82.64 ± 7.35^{b}	78.66 ± 3.96^{b}	75.60 ± 2.35^{b}	72.68 ± 4.44^{b}	70.82 ± 2.11^{b}	20.26 ± 1.77^{b}	19.86± 1.74^{b}	17.35± 1.33^{b}	15.94± 1.71^{b}	14.12± 1.42^{b}	0.35 ± 0.01^{a}	0.41 ± 0.01^{a}	0.41 ± 0.01^{a}	0.27 ± 0.01^{a}	0.28± 0.01^{a}
Group 5	90.12 ± 3.64^{a}	90.22 ± 3.58^{a}	89.23± 3.66^{a}	88.10 ± 2.35^{a}	84.75± 3.12^{a}	32.40 ± 2.33^{a}	30.23 ± 3.54^{a}	28.15 ± 2.88^{a}	26.94 ± 1.85^{a}	25.45 ± 1.35^{a}	0.34 ± 0.01^{a}	0.29 ± 0.01^{b}	0.29± 0.01^{b}	0.26 ± 0.01^{a}	0.24± 0.01^{a}
Group 6	84.85 ± 6.54^{b}	83.61 ± 5.10^{b}	78.47± 7.86^{b}	72.24 ± 4.60^{b}	70.32 ± 5.42^{b}	21.34 ± 3.56^{b}	20.45± 4.02^{b}	19.22± 2.54^{b}	17.28± 3.12^{b}	15.86± 4.22^{b}	0.33 ± 0.01^{a}	0.41± 0.01^{a}	0.43± 0.01^{a}	0.26 ± 0.01^{a}	0.33 ± 0.01^{a}

Values are expressed as Mean±SE; The means with different superscripts in the same column indicate significantly different, ($p<0.05$).

Table 7: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and /or florfenicol (30 mg kg^{-1}) for five successive days on serum total proteins, serum albumin and serum globulin values of experimentally infected chickens with *E. coli* (n=5).

Parameters	Serum total protein (g dl^{-1})					Serum albumin (g dl^{-1})					Serum globulin (g dl^{-1})				
	During treatment			Post treatment		During treatment			Post treatment		During treatment			Post treatment	
Groups	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day
Group 1	4.33 ± 0.21^{a}	4.70 ± 0.10^{a}	4.79 ± 0.10^{a}	4.71 ± 0.12^{a}	4.72 ± 0.14^{a}	1.82 ± 0.08^{a}	1.85 ± 0.05^{a}	1.80 ± 0.02^{a}	1.82 ± 0.02^{a}	1.86 ± 0.02^{a}	1.95± 0.02^{a}	1.96± 0.02^{a}	2.01± 0.02^{a}	2.11± 0.02^{a}	2.14± 0.05^{a}
Group 2	2.66 ± 0.05^{c}	2.86 ± 0.07^{c}	2.85 ± 0.11^{c}	2.89 ± 0.10^{c}	2.82 ± 0.12^{c}	0.84 ± 0.01^{b}	0.81 ± 0.02^{c}	0.80 ± 0.00^{c}	0.81 ± 0.01^{c}	0.80 ± 0.01^{c}	1.74 ± 0.08^{b}	1.67 ± 0.08^{b}	1.62 ± 0.02^{c}	1.66 ± 0.04^{b}	1.64 ± 0.01^{c}
Group 3	2.94 ± 0.02^{b}	3.14 ± 0.01^{b}	3.32 ± 0.04^{b}	3.75 ± 0.01^{b}	4.12 ± 0.13^{b}	1.23 ± 0.01^{b}	1.35 ± 0.01^{b}	1.55 ± 0.07^{b}	1.80 ± 0.04^{b}	1.94 ± 0.00^{b}	1.76 ± 0.08^{b}	1.77± 0.02^{b}	1.92± 0.04^{b}	2.06± 0.09^{a}	2.12± 0.03^{a}
Group 4	2.98 ± 0.01^{b}	3.09 ± 0.02^{b}	3.24 ± 0.04^{b}	3.52 ± 0.11^{b}	4.07 ± 0.14^{b}	1.27 ± 0.01^{b}	1.44 ± 0.04^{b}	1.56 ± 0.03^{b}	1.87 ± 0.02^{b}	1.90 ± 0.05^{b}	1.77 ± 0.08^{b}	1.74± 0.03^{b}	1.85± 0.03^{b}	2.08± 0.02^{a}	2.12± 0.02^{a}
Group 5	2.56 ± 0.04^{c}	2.85 ± 0.11^{c}	2.84 ± 0.02^{c}	3.05 ± 0.10^{bc}	3.13 ± 0.11^{c}	0.83 ± 0.01^{c}	1.00 ± 0.01^{c}	1.04± 0.02^{c}	1.10 ± 0.01^{c}	1.12 ± 0.05^{c}	1.73 ± 0.03^{b}	1.77 ± 0.07^{c}	1.67 ± 0.01^{c}	1.76 ± 0.02^{b}	1.84 ± 0.03^{b}
Group 6	2.97 ± 0.13^{b}	3.44 ± 0.11^{b}	3.55 ± 0.10^{b}	3.74 ± 0.11^{b}	4.16 ± 0.11^{ab}	1.25 ± 0.02^{b}	1.46 ± 0.05^{b}	1.64 ± 0.00^{b}	1.74 ± 0.06^{b}	1.89 ± 0.04^{b}	1.71 ± 0.04^{b}	1.74± 0.02^{b}	1.87± 0.04^{b}	2.00± 0.04^{a}	2.12± 0.02^{a}

Values are expressed as Mean±SE; The means which carry different letters in the same column were significantly different, ($p<0.05$).

Table 8: The effect of oral administration of pefloxacin (10 mg kg^{-1}) and /or florfenicol (30 mg kg^{-1}) for five successive days on serum uric acid and serum creatinine values of experimentally infected chickens with *E. coli* (n=5).

Parameters	Serum uric acid (mg dl^{-1})					Serum creatinine (mg dl^{-1})				
	During treatment			Post treatment		During treatment			Post treatment	
Groups	2nd day	4th day	5th day	7th day	14th day	2nd day	4th day	5th day	7th day	14th day
Group 1	5.12± 0.14^{c}	5.10± 0.16^{c}	5.54 ± 0.25^{c}	5.34 ± 0.15^{c}	5.36 ± 0.12^{b}	1.32± 0.05^{b}	1.29± 0.02^{c}	1.26± 0.08^{b}	1.18± 0.02^{b}	1.16± 0.04^{b}
Group 2	9.26 ± 0.13^{a}	8.46 ± 0.16^{a}	8.66 ± 0.15^{a}	8.74 ± 0.14^{a}	8.74 ± 0.36^{a}	2.18 ± 0.04^{a}	2.13 ± 0.09^{a}	2.15 ± 0.06^{a}	2.11 ± 0.04^{a}	2.14± 0.05^{a}
Group 3	7.33 ± 0.15^{b}	7.44 ± 0.24^{b}	6.36 ± 0.25^{b}	6.47 ± 0.11^{b}	5.77 ± 0.13^{b}	1.19 ± 0.05^{b}	1.85± 0.02^{b}	2.51± 0.08^{a}	2.22± 0.09^{a}	1.35± 0.04^{b}
Group 4	7.44 ± 0.15^{b}	7.66 ± 0.16^{b}	6.62 ± 0.31^{b}	6.48 ± 0.41^{b}	5.82 ± 0.21^{b}	1.24 ± 0.07^{b}	1.81± 0.04^{b}	2.59± 0.03^{a}	2.26± 0.01^{a}	1.41± 0.02^{b}
Group 5	7.32 ± 0.24^{b}	7.22 ± 0.28^{b}	7.53± 0.26^{b}	6.88 ± 0.15^{b}	6.75± 0.22^{b}	1.22 ± 0.03^{b}	1.80 ± 0.04^{b}	2.57 ± 0.08^{a}	2.19 ± 0.05^{a}	1.47 ± 0.05^{b}
Group 6	7.35 ± 0.24^{b}	7.61 ± 0.13^{b}	6.47± 0.46^{b}	6.24 ± 0.66^{b}	5.32 ± 0.12^{b}	1.25 ± 0.06^{b}	1.89± 0.02^{b}	2.56± 0.04^{a}	2.24± 0.02^{a}	1.54± 0.02^{b}

Values are expressed as Mean±SE; The means which carry different letters in the same column were significantly different, ($p<0.05$).

CONCLUSION

From the above-mentioned results in this study, it could be concluded that oral administration of pefloxacin, (10 mg kg^{-1}) and florfenicol (30 mg kg^{-1}) combination for 12 hours for five successive days is very effective in controlling of colisepticemia in broiler chickens and minimize the possibility of formation of bacterial resistance. Also, the possible side effects due to both treatments are short-term as the most of the promoters went back to normal after two weeks post drugs administration.

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