Evaluation of cefquinome’s efficacy in controlling avian colibacillosis and detection of its residues using high performance liquid chromatography (HPLC)

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Abstract

This study aimed to evaluate the efficacy of cefquinome in treatment and controlling of Escherichia coli experimentally infected broiler chickens, in addition of detection of its residues using High performance liquid chromatography (HPLC). In this study, 150 one-day old Cobb broiler chicks were used. On the 14th day chicks experimentally infected and divided into 3 equal groups (50 each); control group (G1) non-infected, non-treated, (G2) infected with E. coli O78 non treated, (G3) infected with E. coli O78, cefquinome treated. Cefquinome was administrated 5th day post infection, intramuscularly by a dose of (2 mg/ kg b. wt) for 3 consecutive days. Experimental E. coli infection in broilers induced weakness, loss of appetite, depression, cough and watery diarrhea in addition to a recorded mortality (30%) with reduction in growth performance, erythrogram, total proteins, albumin, antioxidants and haemagglutination inhibition (HI) titers. In addition, a significant increase in feed conversion rate (FCR), leukocytic count, liver enzymes, kidney functions, total globulins, malondialdehyde, nitric oxide and lysozyme activity. Treatment with cefquinome led to decreased mortality rate, improvement in clinical signs, growth performance and modulated most of these altered parameters. Cefquinome’s residues was not detected in breast muscles 3rd day and liver and kidneys 7th days post treatment. Therefore, it’s recommended that cefquinome is a good choice for controlling of colibacillosis in broilers and its withdrawal time 3 days in breast muscles and 7 days in liver and kidney post treatment.

1. Introduction

Chicken meat provides an animal protein of high biological value, where it contain all the essential amino acids required for growth with unsaturated fatty acids and low cholesterol value (Abou Hussein, 2007). Avian pathogenic Escherichia coli is the causative agent of colibacillosis (Gross, 1991). E. coli, which belongs to family Enterobacteriaceae, is a Gram-negative, rod shaped, non-spore forming bacilli and facultative anaerobe, which causes high mortalities and economic loses in poultry industry (Abd El-Tawab et al., 2015). Some factors like poor ventilation, overcrowding, dehydration and extremes of temperature affecting the occurrence of colibacillosis (Kaul et al., 1992). Lutful (2010) reported that broilers affected with E. coli showed weakness, loss of appetite, depression, cough, sneezing and watery diarrhea, which were more prominent clinical symptoms with morbidity and mortalities.

Gross pathological lesions of colibacillosis infection cause a gross pathological lesions as air saculitis, peritonitis, osteomyelitis, enteritis, pericarditis, hemorrhage and congestion in liver, kidneys, spleen and other parenchymatous organs (Abd El-Tawab et al., 2015).

Cephalosporins were widely used in veterinary medicine because of their broad-spectrum activity and safety, as they were
used for treatment respiratory, urinary and genital infections (Greene and Watson, 2001). Cefquinome is one of the fourth generation cephalosporins, mainly used in veterinary medicine (Papich, 2014; Smiet et al., 2012).

Cefquinome has a broad-spectrum in vivo and in vitro activity against Gram positive and Gram negative bacterial species with great resistance against β-lactamase (Limbert et al., 1991). Like other β-lactam antimicrobials, they inhibit bacterial cell wall formation by interfering with penicillin binding proteins (PBPs), it composed of a β-lactam ring and a six-membered dihydrothiazine ring which is essential for their antibacterial activity (Prescott, 2013).

A quaternary ammonium side chain attached to the C-3 position in cefquinome, make it differ from the third generation, it can easily penetrate the biological membranes and porins of the bacterial cell wall due to its zwitter ionic property thus enhancing its spectrum activity and bioavailability compared with the second and third generation cephalosporins (Thomas et al., 2006). Cefquinome is resistant to inactivation by β-lactamases due to addition of a methoxyimino-aminothiazolyl moiety into the acyl side chain which improve its antimicrobial potency and extensive antibacterial spectrum (Marshall and Blair, 1999; Neu, 1982). Fourth generation has free radical scavenging potential and good stability against enzymatic hydrolysis (Soejima et al., 2006). Cephalosporins protect against hypochlorous acid (HOCI) oxidative damage. This defense is a consequence of a direct drug scavenging capacity towards HOCI (Lapenna et al., 1995).

Small metabolites of medicinal products or chemical substances that may accumulate within the tissues or edible parts of treated animals are called drug residues (EC-European Commission, 2012).

The aim of this study was to evaluate the efficacy of cefquinome in treatment of E. coli infection in broiler chickens, with focus on its effects on growth performance, hematobiochemical, oxidative status and immunological profile. In addition, determination of its residues using high performance liquid chromatography (HPLC) and its withdrawal times in different broiler tissues (breast muscles, liver and kidney).

2. Material and methods

2.1. Reagents for mobile phase in HPLC:

Tri-floro acetic acid (TFA) was spectrophotometric grade ≥ 99.9% (ALDRICH), Acetonitrile (ACN), de-ionized water (D.W)

2.2. Reagents for tissue sample extraction

Ammonium acetate buffer 0.05 M; pH5, Isooctane, Methanol.

2.3. Instrument and chromatographic conditions

The HPLC system (Agilent Technologies, 1200 series Japan) consists of a quaternary pump and a degasser to pump the mobile phase, an auto-sampler and a column oven. The detection by using a multi-wave detector (MWD) set at 267 nm. The column temperature was kept at 40 °C. The reverse-phase chromatography was performed with an analytical Agilent C18 column (250 mm by 4.6 mm; internal diameter, 5 μm; Agilent Technologies) The HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France).

2.4. Drug and treatment

Cefquinome sulphate (50 ml cobactan)® 2.5%, each 1 ml contain 25 mg cefquinome. It was obtained from Pharma Sweed-Egypt Company and was intramuscularly injected with a dose (2 mg/kg body weight) once daily for 3 successive days (El-Sayed et al., 2015).

2.5. Chicks

A total of 150, 1-day old Cobb broiler chicks were reared in litter system under hygienic measures. Chicks were fed commercial ready-made ration obtained from Feed Mix Company. All experimental chickens were used according to the Committee of Animal Welfare and Research Ethics (protocol ZU-IACUC/2/F/166/2021) in Zagazig University, Egypt.

2.6. Vaccines and vaccination

All chicks were vaccinated with Newcastle disease virus (NDV) (Hitchner B1 on 7th day and LaSota on 18th day of age).

- ND vaccines: Hitchner B1 and LaSota live virus vaccines were obtained from Intervet Boxmeer Company, Vial containing 1 × 10⁶ EID₅₀ Newcastle disease virus/dose, was dissolved in physiological saline (30 ml per 1000 doses) as eye drops.

2.7. Experimental infection:

Escherichia coli O₁₉ was obtained from Animal Health Research Institute, EL-Dokki, Cairo. At 14th day of age, each bird in the challenged groups was orally inoculated with 1 ml of saline containing 1 × 10⁶ colony forming unit (CFU) of E. coli/ml (El-Boushy et al., 2006).

2.8. Experimental design

On day 14th of age, chicks were experimentally infected with E. coli O₁₉ and divided into 3 groups (50 chicks each) randomly. Group (G1) non-infected, non-treated (control), (G2) infected with E. coli and non treated, (G3) infected and treated with cefquinome. Treatment was started after 5 days of experimental infection for 3 consecutive days (19–21 days of age) intramuscularly. Chicks were observed daily throughout the whole experimental period. Body weights were recorded to calculate the weight gain. Clinical signs, feed intake, feed conversion rate (FCR) and mortality rate were recorded as well.

2.9. Sampling

2.9.1. Blood samples

Two blood samples from the wing vein of 3 birds of each group at 7 and 14 days post treatment (28th day and 35th day of age respectively). The 1st sample was taken with anticoagulant for measuring erythrogram and leukogram (total erythrocytic, leukocytic and differential leucocytic count) (Natt and Herrick, 1952), Packed cell volume (PCV) (Cohen, 1967) and hemoglobin content (Wintrobe, 1967). The 2nd blood sample collected without anticoagulant for serum separation for measuring aspartate aminotransferase and alanine aminotransferase (AST-ALT) (Tietz, 1976), Alkaline phosphatase (ALP) (Belfield and Goldberg, 1971), serum total protein (Henny, 1974), serum uric acid (Weissman et al., 1974), creatinine (Henny, 1974), super oxide dismutase (SOD) (Nishikimi et al., 1972), glutathione peroxidase (GPX) (Paglia and Valentine, 1967) and malondialdehyde (MDA) (Draper and Hadley, 1990). Serum protein fractions were performed using cellulose acetate electrophoresis test (Davis, 1964), Hemagglutination inhibition (HI) test for estimation titer of ND (Villasag, 1991). Nitric oxide (Green et al., 1982) and lysozyme activity (Schultz, 1987) measured at 1st and 3rd day post treatment.
2.9.2. Tissue samples

Three birds was slaughtered at 3rd, 5th, 7th days post last dose of cefquinome treatment, 1 g tissues was taken from liver, kidney and breast muscles and stored at $-20\,^\circ$C until assayed for detection of cefquinome residues using HPLC (Abd Elhafeez and Fadel, 2021).

2.10. Sample extraction

One gram of tissue sample was added in 15 ml poly-propylene centrifugal tube. 4 ml of 0.05 M ammonium acetate buffer (TAC5) pH 5 and 1 ml isooctane, were added, and mixed for few seconds, then centrifuged at $2400 \times g$ 10 min. The isooctane was discarded and the supernatant was transferred to previously activated solid phase cartridges with 1 ml MeOH, 1 ml D.W and 1 ml TAC5; then cartridges was washed using 1 ml TAC5, 1 ml D.W, then 1 ml 10% ACN. Air was passed via the cartridge for 5 min. elution by 1 ml elution solution 20% ACN; at a flow rate of 3 ml/min, the elute was evaporated at $50\,^\circ$C under nitrogen stream till complete dryness, then re-constituted in 500 μl D.W, filtration with 0.45 um Acro-discs before injection into HPLC system (Elazab et al., 2016).

2.11. Calibration curve

The cefquinome calibration standard was prepared at concentrations of 25, 50, 100, 250, 500, 1000, 2500 ppb in blank.

2.12. Statistical analysis

The obtained data were statistically analyzed by a one-way analysis of variance (ANOVA) method followed by Tukey’s HSD multiple comparison test. Differences were considered significant at $p < 0.05$.

3. Results

The E. coli infected non treated group (G2) appeared symptoms of E. coli infection represented by loss of appetite, depression, ruffled feathers and diarrhea with mortality rate 30%. (Fig. 1).

Growth performance parameters were badly affected in the E. coli infected group, as there were a significant decrease in body weight, weight gain and feed consumption with a significant increase in FCR when compared with control group. Cefquinome treatment improve the body weight, weight gain and FCR with reduction in mortalities 6% (Table1).

E. coli infection cause a significant reduction in erythrogram and lymphocytes beside a significant increase in total leukocytic count, neutrophils compared with control group (G1). Cefquinome treated group showed a significant increase in erythrogram (RBCS, Hb, and PCV) and lymphocytes with a significant decrease in total leukocytic count and neutrophile compared with infected non treated group (Table 2).

Infection with E. coli cause oxidative stress by significant reduction in SOD and GPX with increase in MDA levels compared to control. Meanwhile, oxidative status improved in treated group (Table 3).

On comparison with control group, nitric oxide and lysozymes activity were significantly increased ($P < 0.05$), meanwhile HI titers against Newcastle was significantly decrease in infected non treated broilers, while E. coli infected cefquinome treated group
showed significant decrease in nitric oxide, lysozyme activity and increase in HI titers compared to G2 (Table 4).

Infection with E. coli cause significant reduction in total protein, albumin level and A/G ratio, while total globulins and γ globulin was significantly increased (P < 0.05) with non-significant change in α and β globulin compared to the control. Cefquinome treatment of the infected broilers significantly improve these parameters toward the control levels (Table 5).

Liver enzymes and kidney function were significantly (P < 0.05) increased in E. coli infected group, cefquinome treatment improve these functions (Table 6).

### 3.1. Standard curve concentration

Cefquinome calibration curve was prepared at concentrations of 25, 50, 100, 250, 500, 1000, 2500 ppb respectively. Linearity existed within the range of 25 ppb and 2500 ppb with a correlation coefficient (r²) of 0.99921. The retention time (R.T.) of cefquinome was 13.745 min (Table 7), (Figs. 2-3).

Cefquinome's residues were highly concentrated in kidney followed by liver and not detected in breast muscles at 3rd day post treatment. On 7th day post treatment, it completely not detected in liver and kidney tissues of broiler (Table 8), (Fig. 4).

### 4. Discussion

The current study applied for evaluation efficacy of cefquinome in treatment E. coli infected broilers by its effects on growth performance, hematobiochemical, oxidative status and immunological parameters with detection of its residues in different tissues of boilers.
The clinical signs as loss of appetite, depression, respiratory symptoms including sneezing, gasping, mild conjunctivitis and diarrhea of colibacillosis with high mortalities in G2 because of excretion of endotoxins by *E. coli* (Roushdy, 2007; Mabrouk, 2016).

Medication of broilers infected with *E. coli* using cefquinome improve the general health status, growth performance with disappearance of clinical signs (El-Gendy et al., 2009; Nasr El Deen et al., 2015).

In this study, decreased body weight, weight gain and increased feed conversion rate throughout the experimental period were clearly appeared in infected broilers, which might be attributed to intestinal damage and poor digestion caused by microorganisms; these results were agreed with (Mabrouk, 2016; Fadl et al., 2020). Cefquinome treated group showed decreased feed conversion compared with non-treated, this previous data is supported by (Radwan and Radi, 2010).

Alexander (1985) reported that cefquinome cause increase nutrients absorption leading to improving in weight gain. Reduction in erythrocyte parameters caused by *E. coli* infection could be attributed to bacterial endotoxins, which cause intravascular destruction of erythrocytic cells and consequently lead to hemolysis with breakdown of hemoglobin (Karaivanov, 1984).

*E. coli* produces cell damaging protein toxin that causes changes in cell membrane permeability and formation of surface lesions causing RBCs destruction (Dagmar et al., 2002). In addition, (Tserenpuntsag et al., 2005) stated that *E. coli* lipopolysaccharide

### Table 5
The effect of cefquinome on serum total protein, albumin, globulin and A/G ratio of *E. coli* experimentally infected broiler chickens. (Mean ± S.E) (n = 3).

| Group | Serum total protein (g/dl) 7th day post-treatment | Albumin (g/dl) 7th day post-treatment | Globulin (g/dl) 7th day post-treatment | A/G ratio 7th day post-treatment | Serum total protein (g/dl) 14th day post-treatment | Albumin (g/dl) 14th day post-treatment | Globulin (g/dl) 14th day post-treatment | A/G ratio 14th day post-treatment |
|-------|-----------------------------------------------|----------------------------------|---------------------------------|-------------------------------|-----------------------------------------------|----------------------------------|---------------------------------|-------------------------------|
| G1    | 5.93 ±0.19                                    | 2.85 ±0.03                       | 3.08 ±0.16                     | 1.24 ±0.01                    | 0.93 ±0.01                                    | 0.9 ±0.15                        | 0.93 ±0.04                     | 2.68 ±0.05                    |
| G2    | 4.76 ±0.07                                    | 1.14 ±0.04                       | 3.62 ±0.03                     | 1.81 ±0.02                    | 0.92 ±0.02                                    | 0.89 ±0.03                       | 0.91 ±0.01                     | 1.30 ±0.05                    |
| G3    | 5.37 ±0.04                                    | 2.11 ±0.06                       | 3.26 ±0.03                     | 1.42 ±0.01                    | 0.95 ±0.02                                    | 0.89 ±0.05                       | 0.93 ±0.02                     | 2.56 ±0.01                    |

### Table 6
The effect of cefquinome on liver and kidney function tests of *E. coli* experimentally infected broiler chickens. (Mean ± S.E) (n = 3).

| Group | Liver enzymes (U/L) 7 days post-treatment | Liver enzymes (U/L) 14 days post-treatment | Kidney function (mg/dl) 7 days post-treatment | Kidney function (mg/dl) 14 days post-treatment |
|-------|------------------------------------------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|       | ALT                                       | AST                                       | ALP                                           | Creatinine                                    | U.Acid                                      |
| G1    | 48.07 ±0.34                               | 46.32 ±0.49                              | 235.02 ±0.4                                  | 0.99 ±0.01                                    | 5.39 ±0.11                                  |
| G2    | 64.52 ±0.2                               | 61.39 ±0.15                               | 303.63 ±0.79                                 | 1.92 ±0.01                                    | 7.95 ±0.02                                  |
| G3    | 56.57 ±0.23                               | 48.8 ±0.49                               | 244.61 ±0.22                                | 1.37 ±0.02                                    | 5.52 ±0.01                                  |

### Table 7
Area under the curve corresponding to standard cefquinome concentrations.

| Retention time | Level | Amount(ppb) | Area | Resp. Factor |
|----------------|-------|-------------|------|--------------|
| 13.745 min     | 1     | 25.000      | 5.601| 4.464        |
|                | 2     | 50.000      | 10.919| 4.579        |
|                | 3     | 100.00      | 21.533| 4.644        |
|                | 4     | 250.000     | 56.581| 4.418        |
|                | 5     | 500.000     | 112.270| 4.454        |
|                | 6     | 1000.000    | 220.060| 4.544        |
|                | 7     | 2500.000    | 552.120| 4.528        |

![Fig. 2. Calibration curve of cefquinome.](image-url)
has direct effect as it inhibits bone marrow cells and its nephrotoxicity decrease erythropoietin in blood. Arhoumah (2018) reported that cefquinome has bactericidal activity and improve erythrogram.

Leukocytosis was detected in chickens suffering from colibacillosis could be due to tissue destruction (Coles, 1967), this results agreed with (Nasr El Deen et al., 2015) in broilers and (Tharwat et al., 2013) in turkey.

Arhoumah (2018) and Shawky (2006) reported that cefquinome administration decrease the elevated TLC in infected broilers.

Malondialdehyde (MDA) reactive aldehydes, because of lipid peroxidation indicating oxidative stress (Mandal et al., 2015).

Imbalance between oxidants production and organism’s respective defense systems defined as oxidative stress (Berridge et al., 1996).

In this study, E. coli infection induced an elevation in MDA level in infected group related to excessive production of free radicals causing stress and cellular toxicity, this results agreed with (Fadl et al., 2020) who found high levels of MDA in E. coli infected broilers.

Accumulation of reactive oxygen species (ROS) and oxidant/antioxidant imbalance will lead to decrease levels of SOD and GPX in infected chicks (Kilany et al., 2018).

Treatment with cefquinome improve the antioxidant levels as according to (Soejima et al., 2000). Cephalosporins reduce hepatic oxidative damage, as they are thioether having free radical scavenging properties by preventing the free radical-mediated oxidation of sulfhydryl group. Its antioxidant defense activity related to protection against HOCl-driven oxidative injury (Lapenna et al., 1995).

Lysozyme are protein found in the body fluids, tissue and cells of living organisms having digestive and defense functions by lysis to the bacterial cell wall (Maraghi et al., 2012). During infection, lysozyme activity increased because of lysozyme gene transcription increased (Lowry et al., 2005; Paulsen et al., 2003) and phagocytosis-induced degranulation (Dey and Bishayi, 2015).

Treatment of E. coli infected broiler chickens with cefquinome cause reduction in lysozyme activity; in particular, neutralization of the medium by its acidification properties, which significantly reduces the lysozyme exocytosis, also inhibition of NADPH oxidase needed for activation of lysosomes (Bassler et al., 1982).

Nitric oxide produced by the enzyme nitric oxide synthase from L-arginine, it’s a short highly reactive free radical (Thippeswamy et al., 2006). Herein, E.coli infection remarkably elevate serum nitric oxide, explained by increase of nuclear factor kappa B (NF-κB) leading over release of inflammatory mediators like cytokines and inducible NO synthase (iNOS) causing more NO formation (Macdonald et al., 2003). Tumor necrosis factor alpha (TNF-α) elevation led to increasing NO causing tissue damage (Zhao et al., 2002).

Treated group showed decrease NO level due to fourth generation of cephalosporin able to suppress TNF-α induced by E.coli endotoxin consequently led to decrease NO (Coleman, 2001).

The reduction in HI titer of ND in infected group was returned to changes in the acid-base balance due to digestive disorders, enteritis and diarrhea (Sil et al., 2002) and down regulatory effect and stress of infection post vaccination (Abd El Tawab et al., 2015; Abd El-Ghany and Ismail, 2014; Hassanin et al., 2014; Hegazy et al., 2010), reported the same results as higher HI titer were detected in healthy vaccinated chickens than other E. coli infected vaccinated.

Table 8

Concentrations of cefquinome (ppb) after repeated intramuscular administration in breast muscles, liver, and kidney of E. coli experimentally infected, cefquinome treated broiler chickens (group 3). (Mean ± S.E) (n = 3).

| Organs       | Residue level (ppb) | 3rd day post treatment | 5th day post treatment | 7th day post treatment |
|--------------|---------------------|------------------------|------------------------|------------------------|
| Brest muscles | ND                  | ND                     | ND                     |                        |
| Liver        | 102.4 ± 0.473       | 35.4 ± 0.252           | ND                     |                        |
| Kidney       | 353.8 ± 0.635       | 55.74 ± 0.399          | ND                     |                        |

ppb: part per billion, ND: not detected.
Cefquinome treated group showed significant increase in HI titer compared with infected one, due to improvement the levels of TNF-α and IL-10 elevated by E.coli infection (Arhoumah, 2018).

E. coli infection cause a significant decreases in total serum protein, attributed to kidney diseases, causing protein loss and congestive heart failure (Blood et al., 1994), albumin level were significantly decreased due to decrease in feed intake, anorexia and hepatic damage (Deshmukh, 2006), these results were agreed with that of (EL-Nemr, 2011; Godbole, 2017; Manafi et al., 2016; Zaki et al., 2012). Also, E. coli infection cause significant increase in globulins, as a result of liver cirrhosis, hepatitis, and Kupffer cell proliferation (Sharma et al., 2015), this result reinforced with (Fadl et al., 2020). γ-globulins elevation in infected group associated with immune system activation (Gharieb and Youssef, 2014) this results agreed with (Hashem et al., 2020).

Cefquinome treatment of E. coli infected broiler chickens improved proteinogram compared with infected non treated group (Elkomy et al., 2019).

Infected broiler chickens showed increase in liver enzymes, this might be due to the hepatic degenerative changes, causing escape of liver enzymes with high abnormal levels into serum due to alteration of cellular permeability (Joan and Pannel, 1981).

Unger et al. (1989) attributed the increase of liver enzymes in E.coli infection to perfusion of certain microvascular segments and decrease perfusion of others because of redistribution of hepatic microvascular blood flow within the liver lobule. These results were supported by that obtained by (El-Kadeem, 2009; Shawky, 2006).

Degenerative changes in renal tubules and impairment of their function, decreases the excretion of uric acid and creatinine leading to increase its levels in serum in E. coli infected group (Kaneko, 1980). Our results were in accordance with that obtained by (Abdalla and Adayel, 2006; El-Sayed, 2007). Treatment of E. coli infected broiler with cefquinome showed reduction in liver enzymes which agreed with (Elbaz et al., 2020), kidney functions as reported by (Nasr El Deen et al., 2015).

Many countries have monitoring scheme to avoid antibiotic residues in food of animal origin to ensure food safety (Albayoumi, 2015).

In the present investigation, repeated intramuscular administration of cefquinome revealed that kidneys retains the highest drug concentrations followed by the liver in the infected treated broilers while the lowest drug concentrations found in muscles. These findings suggested that cefquinome excreted mainly via the kidney (Limbert et al., 1991; San Martin et al., 1998). These results were agreed with that reported by (El-Sayed et al., 2015; Gaber, 2005) in chickens and (Abd Elhafiez and Fadel, 2021) in rabbits.

5. Conclusion

The repeated intramuscular administrations of cefquinome (2 mg/kg b.wt.) once daily for three consecutive days provides an effective treatment against E. coli infection in broiler chickens. The recommended withdrawal time is 3 days in muscles and 7 days for liver and kidneys in E. coli infected cefquinome treated broilers to be safe for human consumption.

6. Ethics statement

The protocol was conducted according to the Committee of Animal Welfare and Research Ethics (protocol ZU-IACUC/2/F/166/2021) in Zagazig University, Egypt.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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