International Journal of Pharmacology and Toxicology, 5 (1) (2017) 51-56

International Journal of Pharmacology and Toxicology

Website: www.sciencepubco.com/index.php/IJPT
doi: 10.14419/ijpt.v5i1.7193
Research paper

Efficacy of colimox® (a new combination of amoxicillin and colistin) in the control of experimentally induced necrotic enteritis in broiler chickens

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Abstract

The aim of the present study was to determine the efficacy of amoxicillin and colistin either alone or in combination (colimox®) for control of experimentally induced *C. perfringens* infections in broiler chickens. Three hundred birds were used and divided into five groups (A, B, C, D and E, each of 60 bird). At the 14th day of age, birds in all groups (except group A) were inoculated orally with 0.5 mL of *C. perfringens* broth culture (10^9 CFU/mL). Two days later, drugs were orally administered once daily for five consecutive days as follow; Group A and B were left untreated. Group C, D and E were treated with amoxicillin (20 mg/kg b.wt), colistin (100000 IU /kg b.wt), colimox® (amoxicillin plus colistin), respectively. The efficacy of used drugs were determined on the basis of clinical symptoms, mortality rate, body weight, total feed consumption, feed conversion rate and scores of intestinal lesions. The minimum inhibitory concentration (MIC) of amoxicillin against *C. perfringens* was done by micro-broth dilution method and was ≤ 2 µg/mL. Efficacy results indicated that all used medications were effective (but with different degrees) in control of *C. perfringens* induced necrotic enteritis as expressed by significant ($p<0.05$) reduction of clinical signs, mortalities and intestinal lesion scores as well as improving the performance parameters in broiler chickens, however colimox® was the superior. It could be concluded that combination of amoxicillin and colistin (colimox®) was of a considerable value in control of necrotic enteritis in broiler chickens than using them separately.

Keywords: *Amoxicillin; Colistin; Efficacy; C. Perfringens; Broiler Chickens.*

1. Introduction

Prevention of diseases is a major concern in poultry industry, due to resulting decreased growth and increased mortality (Porter 1998). Necrotic enteritis (NE) is considered one of the most economically important diseases, causing major economic losses in poultry industry and seriously affecting the performance of birds (Skinner et al. 2010). NE is an acute clostridium infection characterised by sudden onset, high mortality and severe necrosis of intestinal mucosa. NE in chickens which was first reported by Parish (1961), is an enteric disease caused by *C. perfringens* type A, a Gram-positive anaerobic spore-forming, rod-shaped bacterium (Baba et al. 1997, Nauerby, et al. 2003). NE in chickens induces major production losses due to increased mortality (McDevit et al. 2006) and decreased feed conversion rate and weight gain.

C. perfringens is the most important clostridial pathogen of poultry, causing avian malignant disease, gizzard erosions, and gangrenous dermatitis (Sasaki et al. 2000, Brennan et al. 2003, Kwon et al. 2004, Lovland et al. 2004, Thompson et al. 2006). *C. perfringens* is a normal inhabitant of intestinal tract of chickens as well as a potential pathogen causing necrotic enteritis (Van Immerseel et al. 2004). It is characterized by sudden onset of diarrhea and mucosal necrosis (Skinner et al. 2010).

Antimicrobial therapy is an important tool in reducing enormous losses in poultry industry caused by bacterial infection (Gazdzinski & Julian 1992). Necrotic enteritis can be prevented by use antibiotics (Brennan et al. 2001). Among these antimicrobials amoxicillin and colistin. Amoxicillin is a one of most effective β lactam antibiotic (Palmer et al. 1976). It is widely used in veterinary medicine because of its broad-spectrum antimicrobial activity, good, rapid absorption (61% bioavailability, T_{max} 0.5–1 h, Sumano Lopez & Gutierrez Olivera 2010) and good penetration into tissues (Amin et al. 1994). Its bactericidal effect is done by inhibiting the biosynthesis of cell wall mucopeptide during bacterial multiplication (Nagaralli et al. 2002). Previous studies reported that *C. perfringens* strains were susceptible to amoxicillin *in vitro*, (Martel et al. 2004).

Colistin is a highly selective antibiotic for prevention and treatment of infectious enteritis caused by Gram–negative bacteria, especially *Salmonella, Colibacillus, Pseudomonas, Shigella, Heamophilus* and *Aerobacteria* in poultry and large animals. Colistin sulfate is not absorbed from intestine thus producing no residues in various tissues of target animals. Colistin sulfate maintains its potency in intestinal tract even in presence of food, digestive ferments, enzymes, pus and necrotic tissues. Therefore, Colistin is an ideal drug for treating a variety of the gastrointestinal tract infections.

Since colistin sulfate is a narrow spectrum antibiotic, it was frequently used as a combination therapy in order to broaden the antibacterial activity. Synergistic effects were reported in different studies to examine the combination of colistin with other antibiotics (Petrosillo et al. 2008, Nation & Li 2009, Gordon et al. 2010). A recent study (Liang et al. 2011) demonstrated that the combination of colistin/meropenem as well as colistin/minocycline and colistin/rifampicin were synergistic *in vitro* against extensive drug-resistant *A. Baumannii*. Souli et al. (2009) demonstrated a

synergistic activity of colistin and imipenem as a combination therapy against *K. pneumonia* isolates. Another study (Arroyo et al. 2009) showed the synergistic effects of colistin and tigecyline in combination against pan-drug-resistant *A. baumannii* clinical isolates. The combination of colistin with ceftazidime against *P. aeruginosa* (Gunderson et al. 2003), with azithromycin against *P. aeruginosa* (Landman et al. 2005) and with doxycycline against *K. pneumonia* (Elemam et al. 2010) showed a considerable synergism. In a comparative efficacy study with metronidazole and probiotics against *C. perfringens* infection in broilers, colistin sulfate showed also a considerable efficacy (Aslam et al. 2016).

To our knowledge, there are no any published data about colistin sulfate and amoxicillin trihydrate combination for controlling *C. perfringens* infection in broiler chickens. Therefore, the present study was carried out to evaluate the efficacy of amoxicillin and colistin either alone or in combination (colimox®) against experimentally induced necrotic enteritis in broiler chickens. The *in vitro* MIC of amoxicillin against *C. perfringens* were also determined.

2. Materials and methods

2.1. Drugs

2.1.1. Amoxicillin

Amoxicillin is a semisynthetic broad-spectrum penicillin with low toxicity and widely used in many animal species for treatment of respiratory, digestive, skin and other infections (Palmer et al. 1976, Keefe 1977). It was used therapeutically at a dose of 20 mg/kg b.wt. Once daily for 3-5 consecutive days orally or intramuscularly (Brander et al. 1993). Amoxicillin trihydrate was obtained as a pure powder from ATCO Pharma for Pharmaceutical Industries Co., Egypt.

2.1.2. Colistin

Colistin is a mixture of cyclic polypeptides colistin A and B and belongs to class of polypeptide antibiotics known as polymyxins. Colistin is effective against most Gram-negative bacilli. Colistin sulfate is used to treat intestinal infections, or to suppress colonic flora. Colistin sulfate used as water-soluble powder formulations. Its recommended therapeutic dose is 100000 IU/kg. b.wt. orally. It was obtained as a pure powder from ATCO Pharma for Pharmaceutical Industries Co., Egypt.

2.1.3. Colimox®

colimox® is a combined antibacterial product containing amoxicillin trihydrate and colistin sulfate. Each 100 g powder contains 23 g amoxicillin trihydrate (eq. to 20 g amoxicillin base) and 4.16 g colistin sulfate (Eq.to 3.33 g colistin base or 100 M.I.U.). It is manufactured by ATCO Pharma for Pharmaceutical Industries Co., Egypt. In chicken and turkey, it is indicated for treatment and prevention of necrotic enteritis due to *C. perfringens*, enteritis due to *E.coli* and *Salmonella* spp. infections, coli-septicemia and infectious coryza.

2.2. Experimental chicks

Three hundred apparently healthy, one-day-old unsexed Hubbard broiler chicks purchased from a local hatchery and divided into five equal groups, each consists of 60 bird. The birds were kept in thoroughly clean, disinfected pens and were fed *ad libitum* with a commercial starter (1~14 days old), grower (15~29 days old) and finisher (30~42 days old) rations free of antibiotics, coccidiostats, and growth promoters. Water was provided *ad libitum*. The chicks were floor reared in separate units under hygienic measures at chick level. They initially had an environmental temperature of 32°C, reduced 2°C gradually each week. A continuous lightening pattern was used. These chicks were underwent routine vaccination program against Newcastle, Gumboro and avian influenza diseases. All experiments were performed in accordance with guidelines set by the Ethical Committee of Faculty of Veterinary Medicine, Benha University, Egypt.

2.3. Experimental infection

Toxigenic strain of *C. perfringens* type (A) was kindly obtained from microbiology department, Animal Health Research Institute. Dokki, Giza, Egypt. That strain was isolated from a broiler chickens flock suffered from NE. The organism was anaerobically cultured on 10 % sheep blood agar media containing 200 ug/mL neomycin sulfate and incubated at 37°C for 24 h in Gaspack anaerobic jar. Thereafter, the microorganism was cultured in a cooked meat medium and incubated anaerobically at 37°C for 12 h. The obtained culture was centrifuged at 1000 RPM for 10 min, then the concentration of *C. perfringens* was adjusted to a turbidity of opacity tube to 10^9 colony forming unit (CFU)/mL. At the 14th day of age, infection of chickens was carried out in all groups (except group A) by oral inoculations with 0.5 mL of *C. perfringens* broth culture (Dahiya et al. 2005).

2.4. Efficacy study

Birds had not been exposed to any antibacterial agent for 2 weeks prior to the first administration of amoxicillin or colistin and/or colimox®. To minimize absorption variability due to drug-feed interactions, birds were fasted at least 6 h before dosing and water was withdrawn 1 h prior to administration of drugs. After two days of last inoculation (at 16th day of age) where the clinical symptoms of NE were established, drugs were orally (in drinking water) administered once daily for five consecutive days (16th, 17th, 18th, 19th and 20th day of age) as follow; Group A; uninfected untreated. Group B; Infected untreated. Group C; infected and amoxicillin (20 mg/kg b.wt) treated. Group D; infected and colistin (100000 IU /kg. b.wt) treated. Group E; infected and treated with colimox® (20 mg amoxicillin plus 100000 IU colistin/kg b.wt.) corresponding to 1 g of colimox®/liter of drinking water. All chickens (treated and untreated ones) were daily observed during and after challenge and treatments until end of experiment (6 weeks old). Just after appearance of first clinical sign and mortality, the efficacy was evaluated by recording clinical signs score, mortality rate, performance parameters (body weight, body weight gain, feed consumption and feed conversion rate) and postmortem intestinal lesions scores of chickens in each group.

2.4.1. Clinical signs scores

The clinical symptoms appeared after clostridial infection were recorded after the fifth day of treatments (at 20th day of age) and scored from 0 to 3 depending on general behavior of birds (Keyburn et al. 2006). Scores were as follows; 0) bright, active, alert, attracted to feed, normal feces, shiny feather; 1) Less bright, reduced spontaneous activity, less attracted to feed, formed watery feces; 2) Socially isolated but moved when approached; 3) Pronounced lethargy, only moves when stimulated, watery feces, ruffled feather.

2.4.2. Mortality rate

The numbers of dead chickens in infected untreated and infected treated groups were recorded daily during and 48 h after challenge, during and after treatment until end of experiment.

2.4.3. Performance variables

Random chickens of each group were weighed weekly (1st, 2nd, 3rd, 4th, 5th and 6th week of age) and the performance variables, including average body weight, total feed consumption and feed conversion ratio (Wagner et al. 1983) were measured for all groups.

2.4.4. Score of intestinal lesions

Twenty chickens from each of treated and untreated chickens were humanly euthanized by cervical dislocation at 3, 7, 14 and 21 days post challenge. The typical intestinal lesions of *C. perfringens* of dead and euthanized chickens after challenge were recorded and scored according to (Prescott et al. 1978) as following; 0) no gross lesions; 1) thin-walled or friable small intestine; 2) focal necrosis or ulceration; 3) large patches of necrosis; 4) severe or extensive necrosis typical of field cases.

2.5. *In vitro* minimum inhibitory concentrations (MIC)

To investigate the *in vitro* effects of amoxicillin on *C. perfringens* in broiler chickens, the minimum inhibitory concentrations (MIC) of amoxicillin against *C. perfringens* were determined by micro-broth dilution method as described by Brady et al. (1988). The antimicrobial were dissolved in a sterile distilled water to obtain different concentrations of 100, 50, 25, 10, 5, 2.5, 1, 0.5 and 0.1 µg/mL as stock solutions to use with *C. perfringens* (1.5×10^9 organisms/mL) at 37°C for 24 h under anaerobic conditions. At the end of incubation period, tubes were examined visually for turbidity. The tubes with no visible growth indicated the MIC points. MIC was determined as the lowest concentration of amoxicillin that inhibited visible bacterial growth.

2.6. Statistical analysis

Data were statistically analysed by analysis of variance (ANOVA) after analyzing it using the method of Snedecor & Cochran (1982). Differences of $p<0.05$ were considered statistically significant.

3. Results

Healthy chicks (group A, uninfected and untreated) did not show any clinical symptoms of NE throughout experimental period. After experimental infection of broiler chickens (groups B, C, D and E) with *C. perfringens*, the clinical symptoms of NE were evident approximately 36-48 h after infection. The birds were dull, depressed with drooping wings, ruffled feathers, vent pasting, diarrhea, polydipsia, dehydration and with decreased body weight. Severe signs were observed in birds of infected untreated group, whilst severity was decreased gradually in birds of treated groups. The mortality due to *C. perfringens* was significantly ($p<0.05$) decreased in treated groups compared with untreated groups. The mortality rate in infected untreated group was 11.7 % decreased significantly ($p<0.05$) to be 3.33 %, 5 % and 0 % in the amoxicillin, colistin and colimox® treated groups, respectively (Table 1).

Table 1: Effect of amoxicillin (20 mg/kg b.wt.) and colistin (100000 IU/kg b.wt.) given alone or in combination (colimox®) in drinking water for five consecutive days on mortality rate in experimentally infected broiler chickens with *C. perfringens*.

Groups	No. of dead birds	Mortality rate (%)
A (uninfected, untreated)	0/60	0.00^a
B (infected, untreated)	7/60	11.7^c
C (infected, amoxicillin treated)	2/60	3.33^b
D (infected, colistin treated)	3/60	5.00^b
E (infected, colimox® treated)	0/60	0.00^a

Within a column, values followed by different lowercase letters are significantly different ($P<0.05$).

After administration of amoxicillin and colistin either alone or in combination (colimox®) to groups C, D and E, respectively, the clinical symptoms gradually became less severe. In the present study, relief of clinical symptoms of NE after medications were more rapid in group E (colimox® treated) than in group C (amoxicillin treated) than in group D (colistin treated). Clinical signs scores after medications with amoxicillin, colistin and colimox® were recorded in table (2).

Table 2: Effect of amoxicillin (20 mg/kg b.wt) and colistin (100000 IU/kg b.wt.) given alone or in combination (colimox®) in drinking water for five consecutive days on clinical signs score (at 25th day of Age) of healthy and experimentally infected broiler chickens with *C. perfringens*.

Clinical signs and behavior	Score range	Groups				
		A	B	C	D	E
Alertness (normal ~ depressed)	0 - 3	0	3	2	2	1
Attraction to feed (normal to less interest)	0 - 3	0	3	1	1	0
Feces consistency (normal formed watery)	0 - 3	0	3	1	2	0
Feather (normal shiny ~ ruffled broken)	0 - 3	0	3	0	2	1
Cumulative score	-	0	12	4	7	2

Group A; uninfected untreated. Group B; infected untreated. Group C; infected amoxicillin treated. Group D; infected colistin treated. Group E; infected colimox® treated.

The effect after oral administration of amoxicillin, colistin either alone or in combination (colimox®) for five consecutive days on weekly body weight, total feed consumption and FCR in healthy and experimentally infected broiler chicks with *C. perfringens* was displayed in table (3). The data revealed significant ($p<0.05$) differences in the average body weight between treated and untreated groups. The best data were observed in colimox® treated group rather than amoxicillin or colistin treated ones as indicated by the data of FCR. Maximum decrease in weight was into chicks of group B (infected untreated) followed by chicks of group D, C and E, respectively. Birds of group E showed a relatively high body weight (nearly similar to that of group A) as compared to group C and D, respectively. Effects on feed consumption and feed conversion rate were recorded in table (3).

Table 3: Effect of amoxicillin (20 mg/kg b.wt.) and colistin (100000 IU/kg b.wt.) given alone or in combination (colimox®) in drinking water for five consecutive days on body weight, total feed consumption (g) and FCR in healthy and experimentally infected broiler chickens with *C. perfringens*.

	Body weight (g)				
Age (w)	Group A	Group B	Group C	Group D	Group E
1	130.8 ± 5.35^a	128.8 ± 7.24^b	127.6 ± 7.74^a	125.3 ± 9.64^a	124.4 ± 6.25^a
2	350.3 ± 8.93^a	335.3 ± 11.9^b	345.5 ± 9.67^a	351.5 ± 12.6^a	355.2 ± 7.63^a
3	715.5 ± 12.7^a	515.5 ± 12.7^b	695.5 ± 10.8^a	675.8 ± 13.1^a	711.5 ± 10.6^a
4	1120.5 ± 12.5^a	718.6 ± 12.5^b	1100.4 ± 13.3^a	1025.4 ± 14.3^a	1125.0 ± 13.4^a
5	1490.4 ± 15.7^a	985.2 ± 15.7^b	1355.2 ± 17.6^a	1335.2 ± 19.4^a	1389.3 ± 15.7^a
6	1915.5 ± 20.4^a	1315.5 ± 20.4^b	1815.5 ± 20.4^a	1790.5 ± 23.5^a	1890.7 ± 16.3^a
TFC	3800	4100	3900	3980	3850
FCR	1.98	3.12	2.14	2.22	2.03

Group A; uninfected untreated. Group B; infected untreated. Group C; infected amoxicillin treated. Group D; infected colistin treated. Group E; infected colimox® treated;
TFC: total feed consumption (g) per bird; FCR: feed conversion rate.
Data of body weight were represented as Mean ± SEM of 60 broiler chickens per group.
The mean within a raw with no common superscript are considered significantly different.

The intestines of dead and euthanized chickens in infected untreated groups showed variable degrees of friability, thinning and necrosis. The mean intestinal lesion score in healthy and experimentally infected broiler chickens with *C. perfringens* were shown in table (4). Significant ($p<0.05$) reductions in intestinal lesion score were observed in treated chickens as compared with infected untreated ones at various examination intervals. Chicks of group B showed the most severe gross lesions in form of hemorrhages, edema, congestion and necrosis followed by chicks of group D, C, and E, respectively while chicks in group A did not show any gross lesions. Birds in group C showed relatively mild gross lesions compared to group D. The *in vitro* MIC of amoxicillin against *C. perfringens* was ≤ 2 µg/mL.

Table 4: Effect of amoxicillin (20 mg/kg b.wt.) and colistin (100000 IU/kg b.wt.) given alone or in combination (colimox®) in drinking water for five consecutive days on mean intestinal lesion score in *C. perfringens* sacrificed infected and treated broiler chickens

Group	Mean lesion score			
	Days post challenge			
	3	7	14	21
Group A	0.00	0.00	0.00	0.00
Group B	3.27^a	2.59^b	2.05^c	1.6^c
Group C	1.19^a	0.32^b	0.10^b	0.00^b
Group D	1.45^a	0.48^b	0.23^b	0.00^b
Group E	0.67^a	0.12^a	0.08^a	0.00^a

Group A; uninfected untreated. Group B; infected untreated. Group C; infected amoxicillin treated. Group D; infected colistin treated. Group E; infected colimox® treated.
* Within a raw, values followed by different lowercase letters are significantly different ($P<0.05$).

4. Discussion

Although many clinical cases of NE have been recorded in poultry production, including broilers raised on floor (Tsai &Tung 1981), cage-reared layers (Broussard et al. 1986), commercial layers either raised in cages (Dhillon et al. 2004) or in floor pens (Chakraborty et al.1984), there is a significant lack of information in scientific literature about the clinical efficacy of antimicrobials and their combinations for control of NE in broiler chickens.

The current study was conducted to check the efficacy of colimox® a new combination of amoxicillin trihydrate and colistin sulfate against experimentally induced NE in broiler chickens. The experimental birds were divided into five equal groups, each of 60 bird. After 36-48 h of *C. perfringens* infection, the birds of infected groups (B, C, D and E) developed clinical symptoms of NE. The recorded clinical symptoms are agreeable with those obtained by Riddell & Kong (2004), Craven (2000), Opengart & Songer (2013).

Treatment of *C. perfringens* infected broiler chickens with amoxicillin alone or colistin alone or in combination (colimox®) showed an improvement in health status of infected birds but with different degrees as evidenced by gradual disappearance of clinical symptoms after treatment. The clinical signs score of colimox® treated group was smaller than that of amoxicillin treated group, which was smaller than that of colistin treated group (Table 1). This shows that using amoxicillin plus colistin is more effective in relieving clinical signs of NE than using them separately. In addition, amoxicillin is better than colistin in improving clinical signs of NE in broilers infected with *C. perfringens*.

Our results revealed a high mortality rate (11.7%) in infected untreated broiler chicks, decreased to be 3.33 %, 5 % and 0 % in the amoxicillin, colistin and colimox® treated groups, respectively. A nearly similar (12.5%) mortality rate caused by *C. perfringens* in an untreated broilers was obtained by Riddell & Kong (1992) and Abd El-Ghany (2010). The increase in a mortality rate in chickens infected with *C. perfringens* may be due to the effect of bacterial toxins (Sameh et al. 2005). Our results were in accordance with Gharaibeh et al. (2010) who recorded a decreased mortality rate in broiler chickens infected with *C. perfringens* after treatment with amoxicillin. In keeping with these lines, Koutoulis et al. 2015 found that amoxicillin could be effectively used as a curative treatment during an NE outbreak.

In the current study, it has been shown that broiler chickens experimentally infected with *C. perfringens* revealed a significant decline in body weight and increase in feed conversion rate post infection. Growth depressing activity of intestinal *C. perfringens* in chickens has been confirmed (Barnes et al. 1972). Same reduction in body weight gain and elevation in feed conversion rate was recorded by Skinner et al. (2010) who mentioned that, necrotic enteritis resulted in a 12% reduction in body weight, and a 10.9% increase in feed conversion rate compared with healthy birds. An explanation for decrease in body weight in chickens infected by *C. perfringens* was mentioned by Lovland & Kaldhusdal (1999). They found that, clostridial toxins has induced damage in intestinal tissue and liver leading to decrease in nutrients absorption and metabolism, which lead to inferior growth performance of birds.

Our findings revealed a significant improvement in body weight, total feed consumption and FCR in treated groups when compared with infected untreated ones. The obtained data were in agreement with those recorded by Silva et al. (2009) who stated that, healthy chicken received amoxicillin displayed a significant increase in body weight, weight gain and decreased feed conversion rate. Administration of amoxicillin or colistin either alone or in combination for treatment of *C. perfringens* infection resulted in improvement in body weight, total feed consumption and feed conversion rate after medications when compared with infected untreated broiler chicks. This improvement in performance parameters may be due to the antimicrobial effect of the used medications in suppressing growth of *C. perfringens* and decreased its intestinal colonization leading to prevention of necrotic enteritis (Watkins et al. 1997). Our results were also consistent with those recorded by Lanckriet (2010) who stated that the infected broiler chickens by *C. perfringens* and treated with amoxicillin showed an improvement in body weight gain and feed conversion rate. Amoxicillin was effective in abolishing of development of necrotic enteritis (Vissiennon et al. 2000; Sasaki et al. 2001, Koutoulis et al. 2015). Oral administration of colistin was also effective in treatment *C. perfringens* infection in broiler chickens (Aslam et al. 2016).

In the current study, the obtained efficacy data were supported by the findings of Lanckriet et al. (2010). They studied the efficacy of amoxicillin (at 50-150 g/1000 L in drinking water for four days) on the incidence of necrotic enteritis in a subclinical experimental infection model that used coccidiosis as a predisposing factor. They found that amoxicillin was successful curative treatment. Additionally, in a comparative efficacy study with metronidazole and probiotics against *C. perfringens* infection in broilers, colistin sulfate showed a considerable efficacy (Aslam et al. 2016).

The *in vitro* MIC of amoxicillin against *C. perfringens* in current study was ≤ 2 µg/mL. A similar result was obtained by Gad et al. (2011) who found a similar MIC (≤ 2 µg/mL) of amoxicillin against *C. perfringens*. Additionally, MIC of amoxicillin on *C. perfringens* type A isolated from affected intestinal sections in broiler-breeders was less than 2µg/mL (Koutoulis et al. 2015). The antimicrobial susceptibility of *C. perfringens* type A isolated from clinical cases in broiler chickens to various veterinary antibiotics were examined (Gharaibeh et al. 2010). They found that amoxicillin showed a very low MIC < 0.5 µg/mL and concluded that NE infections can effectively be treated with amoxicillin. These findings are also in agreement with Martel et al. (2004) who examined the susceptibility of *C. perfringens* strains from broilers to antibiotics and anticoccidials and reported that *C. perfringens* isolates examined were highly susceptible to amoxicillin with MIC ranging from 0.03 to 0.5 µg/mL.

Although *C. perfringens* is the main cause of NE in poultry, other predisposing factors that alter the media of GIT and create a favorable environment for *C. perfringens* overgrowth are essential to produce both clinical and subclinical types of NE (M'Sadeq et al. 2015). Predisposing factors include elements that directly alter the physical conditions of the gut, either damaging the epithelial lining, increasing mucus production, or altering gut transit times; factors that disrupt the gut microflora; and factors that alter the immune status of birds. Among these factors is the co-infection by toxin-producing microorganisms like *E. coli* and *Salmonella*. These enterotoxins can induce damage of intestinal epithelium that in turn facilitate *C. perfringens* penetration to mucosa. Therefore, in current study, the higher efficacy of colimox® (a new combination of amoxicillin plus colistin) against *C. perfringens* might be due to the higher sensitivity of *C. Perfringens* to amoxicillin as well as the inhibiting effect of colistin sulfate against Gram-negative *enterobacteriacae* that damages the intestinal mucosa and create a favorable environment for *C. perfringens* overgrowth.

5. Conclusions

In conclusion, using colimox®, a new combination of amoxicillin (20 mg/kg b.wt) and colistin (100000 IU/kg b. wt) is a very good medication for treatment and control of necrotic enteritis in broiler chickens.

Acknowledgment

The author wishes to thank Prof. Dr. Ashraf Elkomy (Head of Pharmacology Dept., Fac. of Vet. Med., Benha Univ., Egypt) for his advices on manuscript writing. Furthermore, we thank Dr. Sawsan Elbasiony (Dept. of Poultry diseases, Fac. of Vet. Med., Benha Univ., Egypt) for her help in assessing the lesion scores of infected birds.

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