

Comparative Study of the clinical effects of Levofloxacin and Spiramycin on induced colibacellosis in chicken

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Abstract

The study is mainly focusing to find the sensitivity and efficacy of levofloxacin or Spiramycin on induced colibacellosis in chicken at therapeutic dose for 5 days, Sixty four, 28 day old broiler chicks of mixed sexes were used in the study. They were placed in the animal house at Faculty of Veterinary Medicine, Kufa University. The clinical signs developed after 12 hours in all the inoculated groups. Mortality in all the groups started after 16 -24 h of inoculation. After 12day period the body weight of group treatment with Levofloxacin animals significantly backed to normal values. While animals in group treated with Spiramycin did not indicate improvement in body weight after 12 days of treatment with Spiramycin. The mortality rate in unmedicated group was high as compared to medicated groups, Levofloxacin more effective against Colibacellosis as compared to Spiramycin.

Aim of the study

Our studies evaluate the available therapies, and its effectiveness and safety of levofloxacin and compares' with old antibiotic like Spiramycin.

Key words: *Levofloxacin , Spiramycin, Colibacellosis, chicken.*

دراسة مقارنة للفعالية العلاجية لليفلوفلوكساسين والسبايراميسين للاصابة التجريبية بالعصيات القولونية في الدواجن

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الخلاصة

اجريت هذه الدراسة لمقارنة الفعالية العلاجية لليفلوفلوكساسين والسبايراميسين للاصابة التجريبية بالعصيات القولونية في الدواجن باعطاء جرعة علاجية لمدة 5 ايام متتالية , اجريت هذه الدراسة في كلية الطب البيطري جامعة الكوفة , على 64 طير بعمر 28 يوم , العلامات السريرية ظهرت بعد 12 ساعة , الهلاكات بدأت بعد 16-24 ساعة من الاصابة نسبة الهلاكات في المجموعة السبايراميسين اكثر من اليفلوفلوكساسين , وبعد 12 يوم من العلاج , مجموعة اليفلوفلوكساسين رجعت الى وزنها الطبيعي لذلك فإن اليفلوفلوكساسين اكثر فعالية علاجية ضد العصيات القولونية مقارنة بالسبايراميسين.

Introduction

Colibacillosis is the main disease caused by ExPEC (extraintestinal pathogenic *E.coli*) and has led to millions of dollars in losses to the worldwide poultry industry. Another

problem associated with ExPEC strains is the high rates of antibiotic resistance. Recently, chicken products carry *E. coli* strain O25b can be divided into two groups: commensals and pathogenics. The

pathogenic group is divided into two other subgroups, known as pathogenic *E. coli* and diarrheagenic *E. coli* which has been implicated in gastrointestinal diseases. *E. coli* outbreaks leading to food contamination are often associated with ground beef, and more than (75%) of *E. coli* outbreaks are related to beef.(1),(2).

avian pathogen are known as colibacillosis and with fibrine lesions around organs, as septicaemia, , swollen head syndrome, sinusitis, air sacculite, arthritis,, synovitis, and

cellulitis,. also lead to reduced yield, , and hatching of eggs „quality. poultry serves as the main host for avian pathogen *E. coli*, and undercooked humans infected.

The frequent administration of antibiotics in animal production has provided severe antibiotic resistances to many drugs. This fact leads to great concerns regarding animal and human health. ,(3) The similarities between avian and human *EsxPEC* regarding their virulence genes and phylogenetics backgrounds are related to a great concern of zoonotic risk . Previous studies have demonstrated that healthy poultry and poultry meat from retail markets can be a source of *EsxPEC* for human infections

Levofloxacin is new drug comes to market of Iraq in 2015 as veterinary treatment as the first time , levofloxacin is a synthetic broad-spectrum of fluoroquinolone group and used to treat severe infections which failed to respond to other antibiotics (4),(5). Levofloxacin is active against G- and G+ (6). LEVO used in the treatments of bronchitis, urinary tract infection, pneumonia, soft tissue infections.

Material and methods

* Birds

Sixty four unvaccinated , one day old broiler chicks of mixed sexes were used in the study. The birds were monitored for two weeks for any apparent clinical signs of

disease . The temperature was maintained at $25\pm 2^{\circ}\text{C}$ and humidity at 45–65%. The chickens had free access to water and food without additives, such as antibiotics and growth promoters. These chicks were vaccinated against different diseases

Experimental Design

At the age of 28 days, the chickens were allocated into four equal groups (each of 16):

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

Group 2. Birds were served as infected – non treated

Group 3. Birds were challenged with *E. coli* and treated orally with Levofloxacin (10 mg/kg, B.W), after starting of symptoms once daily for five successive days.

Group 4. Birds were challenged with *E. coli* and treated orally with Spiramycin (15mg/ kg,BW) after starting of symptoms once daily for five successive days.

E. coli strain and inoculation

Escherichia coli strain was provided by poultry pathology section of Veterinary hospital of Kufa ,that was isolated from the diseased air sacs of chick with a field case of colisepticaemia, pathogenic *E. coli* was further sub-cultured for identification and characterized by using the method described by (7) . After finding out the viable cell count, the broth was diluted to have approximately (3×10^8) bacteria per 0.25 ml and was used for inducing the infection. The diluted broth culture of pathogenic strain of *E. coli* having of (3×10^8) bacteria per 0.25 ml was inoculated to all groups intraperitoneal as described by (7).

Morphological and biochemical tests to pathogenic *E. coli*

Biochemical tests

Morphological tests

H₂S

Gram stain

Glucose

Chromagar

Lactose
Eosin Methylen Blue
Sucrose

Mac Conkey agar

Evaluate the clinical effects of the tested medicaments

A-Clinical symptoms, gross lesions and mortality rates:

The Signs, gross lesions, postmortem lesions, morbidity, mortality were noted. The mortality in the infected chicks started and recorded at 72 h after the inoculation of *E. coli*, And after 5 days of treatment .

B- Body weight : the body weight were measured before infection ,and after 6 days and 12 days.

Results

A-Identification of *E .coil* by Cultural and colony characteristics

1- Mac Conkey's agar

The colonies were pin pointed, smooth, glossy and translucent and were rose pink in

color. The size of the colony varied from 2 - 3 mm in diameter after 24 h of incubation at 37°C.

2-Nutrient agar

The colonies were dome shaped, round, convex, colorless and smooth. The size of colonies varied from 1 - 2 mm in diameter after 24 h of incubation at 37°C. In nutrient broth, a slimy deposit was developed in the bottom of the tube which was slight pellicle after 24 h of incubation and by shaking the tube. A uniform turbidity appeared in the tube.

3- Eosin Methylene Blue agar

The colony developed after 24 h of incubation at 37°C were 2 - 3 mm in diameter and exhibited greenish metallic shine by the reflected light and dark purple centers by transmitted light.**Chromeagar:** colony with dark pink to reddish . Figure 4 - 9, 4-10



Figure 1: Chromeagar

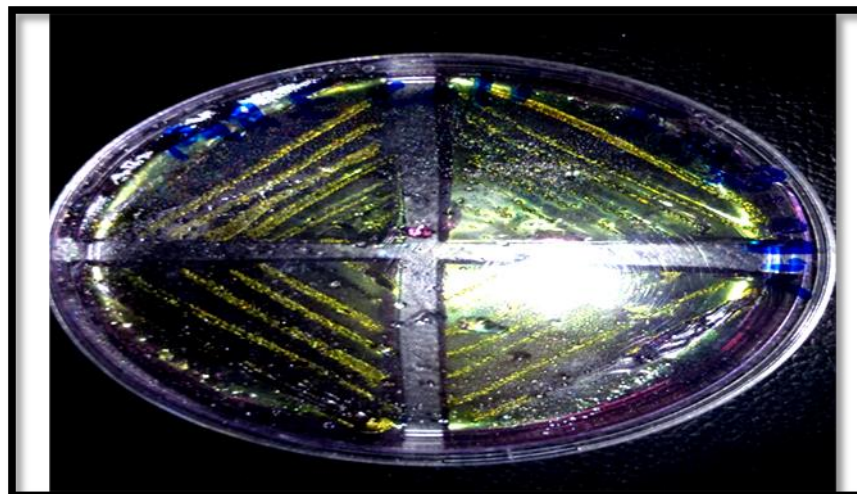


Figure 2: Eosin Methylene Blue agar

B- Staining and motility

The smears were stained with gram's staining and microscopy was performed. All the isolates were Gram negative rods and motile.

C- Biochemical characteristics

Reactions Acid and gas were produced by the fermentation of various sugars like, lactose, glucose and sucrose within one day incubation at 37°C. Methyl red test was performed which was positive, while no hydrogen sulphide was produced.

Clinical signs

The clinical signs developed after 12 hours in all the inoculated groups. Mortality in all the groups started after 16 -24 h of inoculation table(2). The clinical signs and gross pathological lesions observed are rise in temp, inappetence, dullness, depression with closed eyes. Postmortem lesions of the dead birds after 24-72h pericarditis, mild enteritis, congested of lung ,necrotic and hemorrhagic of liver, peritonitis. figure ,(3,4,5,6)

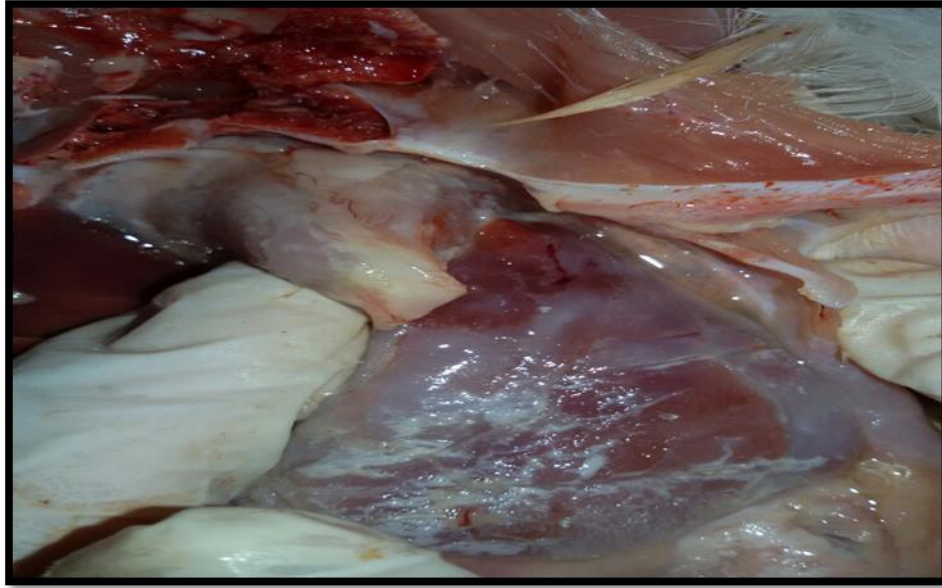


Figure 3: perihepatitis ,pericarditis.

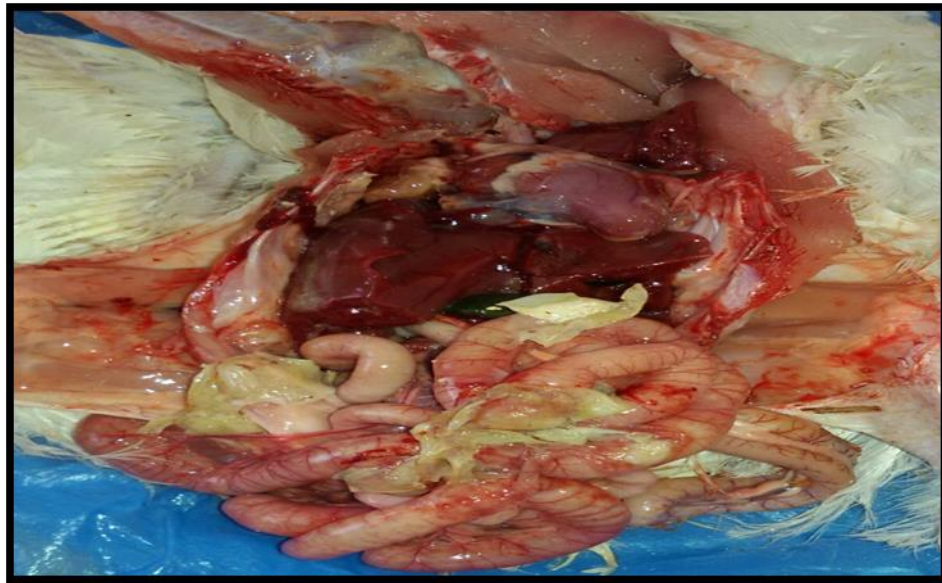


Figure 4: Peritonitis presences of caseous exudates over the abdomen.

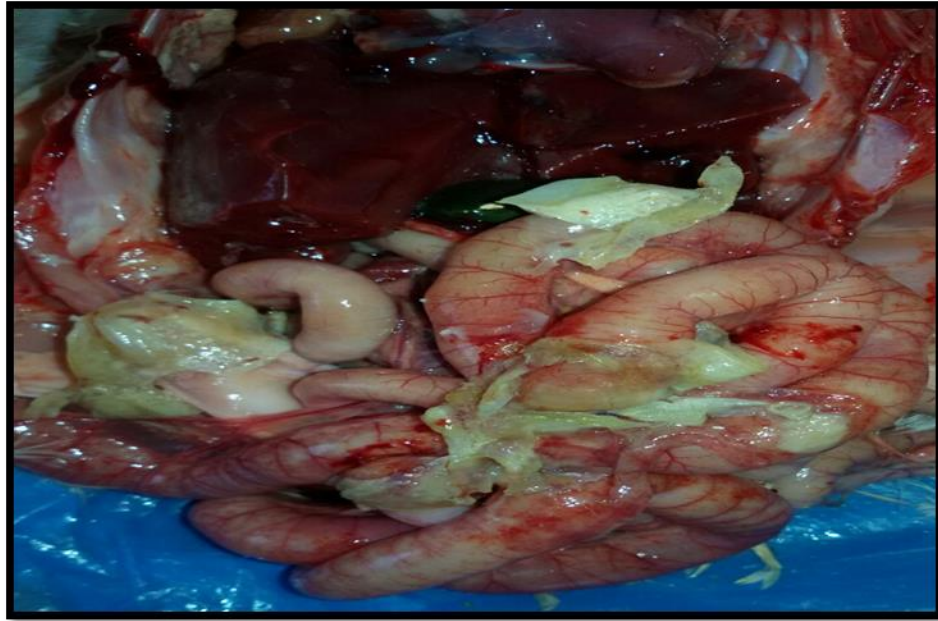


Figure 5: congested of liver presences of caseous exudates over the abdomen .

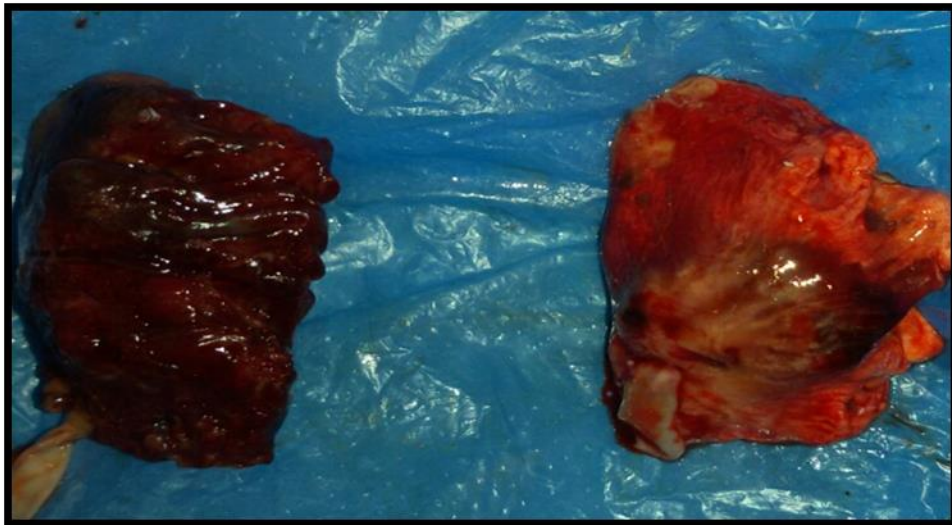


Figure 6: congested of lung

Body weight changes

Variation in birds body weight after 6 day and 12 day after treatment correlation between infection and type of treatment (table1).Body weight decrease significantly ($P<0.05$) after 6 day post infection in all groups when compared with -ve control (group A) which did not display weight loss but gained body weight.

After 12day period the body weight of group C treatment with Levofloxacin animals significantly backed to normal values. While animals in group D treated with Spiramycin did not indicate improvement in body weight after 12 days of treatment with Spiramycin when compared with group C. (table 1).

Mortality rate

Mortality rate in group B reached the highest percentage ,and the total mortality rate was 50% ,while in group C 12.5%,in group D 18.7% table 4-14. The mortality rate in unmedicated group was high as compared to medicated group Levofloxacin more effective against Colibacillosis as compared to Spiramycin. table (2).

Table 1: Weight change (kg) of chickens infected with (*E coli*) and treated orally with Levofloxacin 10 mg/kg B.W or Spiramycin 15 mg /kg for 5 days

Group Period	Group A (1) (-v e control)	Group B(2) (+v e control)	Group C (3) Treated with Levofloxacin	Group D(4) Treated with Spiramycin
Before infected	1.33±0.003 b A	1.39±0.005 C A	1.31±0.005 A A	1.34±0.002 a A
After 6 days treated	1.41±0.005 c A	1.22±0.004 a B	1.30±0.002 b B	1.28±0.008 b B
After 12 days treated	1.73±0.06 c A	1.20±0.001 a C	1.40±0.009 b B	1.33±0.003 b C

Value represent mean $\pm$ S.E, Group no.=16. Different capital letters mean significant ($P<0.05$) results between groups different small letters mean significant ($P<0.05$) results between periods. -ve control: not infected-not treated group; +ve control: infected not treated group.

Table 2 :mortality rate of chicken infected with (*E. coli*) and treated orally with Levofloxacin 10 mg/kg B.W or Spiramycin 15 mg /kg for 5days Group no.=16,-ve control: not infecte not treated group.+ve control: infected not treated group.

Period and Rat Group	After 72h infection	After 5 day treated	Total mortality Rate
	Mortality	Mortality	
Group A (-v e control)	0 %	0 %	0 %
Group B (+v e control)	25%	25%	50%
Group C treated With Levofloxacin	12.5%	0%	12.5%
Group D treated with Spiramycin	18.75%	0%	18.75%

Discussion

Colibacillosis is a common disease that is seen worldwide and is of significant economic importance concerning the loss of livestock (8.) It is the most common infectious bacterial disease of poultry (9) and is seen in cattle , pigs (10) (11) ,and other mammals. Current research is being done to determine if colibacillosis is a public health concern for humans and how antibiotic resistance is affecting the development of a widespread treatment (12).

Colibacillosis is a broad term that refers to any infection or disease caused by the bacteria *Escherichia coli* These infections include colisepticemia, coligranuloma, venereal colibacillosis, coliform cellulitis, peritonitis, salpingitis, orchitis and enteritis, among others In mammals, colibacillosis is usually a primary intestinal or urinary tract infection. While colibacillosis in poultry is usually a secondary disease that occurs when the host's immune system has become

overwhelmed with virulent *E. coli* strains. (13),(14).

Identification of *E .coli* by Cultural and colony characteristics Mac Conkey's agar, chrome agar , Eosin Methylene Blue agar culture media with use for Identification of *E .coli* ,also Biochemical characteristics, fermentation of various sugars like, lactose, glucose and sucrose, Staining and motility :The smears were stained with gram's staining and microscopy was performed. All the isolates were Gram negative rods and motile. Pathogenicity of *E. coli* : pur strain of *E. coli* was injected intra peritoneally in 5 chicks the clinical signs appeared with in 12 hours after the inoculation of *E. coli* and mortality started within 16 - 24 h. our result were in agreement with (15), (16) .

Body weight changes

Body weight decrease significantly ($P<0.05$) after 6 day post infection in all groups when compared with -ve control (group A) not infected-not treated group which did not display weight loss but gained body weight

After 12day period the body weight of group treatment with Levofloxacin animals significantly backed to normal values(1.31 ± 0.005 to 1.40 ± 0.009). While animals in group treated with spiramycin did not indicate improvement in body weight after 12 days of treatment with Spiramycin when compared with group C. treatment with Levofloxacin(1.34 ± 0.002) to (1.33 ± 0.003).

Mortality rate

It was found that levofloxacin is better to control the colibacillosis as compared to spiramycin levofloxacin reduced the mortality rate from (12.5% to 0%) in comparison with that of spiramycin as mortality rate was still high after Spiramycin administration to infected birds(18.75%). The present work will help the veterinarian in diagnosing and prescribing the suitable drug for treating colibacillosis in broiler from the research it may be concluded that *E. coli* infection of birds treated effectively with levofloxacin which is agreed with (17) but with our study Spiramycin not effective on *E. coli* infection because resistant to Spiramycin ,mutants of *Escherichia coli* (18).

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