Animal Health Research Journal Vol. 7, No. 3, September 2019 pp. 15-30

ISSN: 2356-7767

Evaluation of the therapeutic efficacy of difloxacin and florfenicol in different dosage regimen in broiler chickens experimentally challenged by *Klebsiella pneumoniae*

Hesham, S. Taha* and Amany, I. El- Bialy**

Biochemistry, Toxicology and Feed Deficiency Dept., Pharmacology Unit* and Serology Unit**, Animal Health Research Institute, Dokki, Giza, Egypt.

Received in 18/8/2019
Accepted in 18/9/2019

Abstract

This study was conducted to evaluate the efficacy of difloxacin and/or florfenicol in controlling the experimental infection of *Klebsiella pneumonia* in broiler chickens. Seven experimental groups were conducted, **Group I**: non-infected-non- treated, **Group II**: Experimentally infected with *K. pneumonia*e and non-treated, **Group III and Group IV**: infected and treated with difloxacin at a dose of 10 and 30 mg / kg b.wt. once daily for 7 successive days orally respectively, **Group V and VI**: infected and treated with florfenicol at a dose of 20 and 30 mg/ kg b.wt. once daily for 5 successive days orally respectively. **Group VII**: infected and treated with florfenicol (15 mg/ kg b.wt.) and difloxacin (15 mg/ kg b.wt.) once daily for 7 successive days orally.

Difloxacin showed antibacterial activity against *K. pneumoniae* with MIC and MBC values were 0.156 and 0.312 µg/ml respectively while for florfenicol were 3.125 and 6.25 µg/ml respectively. Based on the fractional inhibitory concentration, an indifferent interaction was shown in combination of florfenicol with difloxacin (FIC index = 1.5).

Swabs from Choanal cleft, liver and lung were taken for re-isolation of *K. pneumoniae* and bacterial count. The choanal swabs showed a significant decrease in percentage of the Group IV to 22.2% while being 77.8%, 88.8% and 66.7%, in Groups III, V and VII at 5th day from the first dose respectively. Difloxacin (30mg/kg b.wt.) is superior for the control of *K. pneumoniae* and significantly reduced the bacterial count in liver and lung with log CFU of 2.96 ±0.10 and 3.35± 0.02 followed by group VII was 3.40±0.02 and 3.48±0.01then group III was 3.49±0.05 and 3.54±0.01compared with the control positive group (3.57±0.02 and 3.59±0.01) at 5th day after cessation of drug respectively. Residues of Florfenicol (30mg/kg b.wt.) and difloxacin (30mg/kg b.wt) were detected in liver (0.46±0.06 µg/gm and 0.47±0.06 µg/gm) and lung (0.8 ±0.10 µg/gmand 0.58 0.03 µg/gm) after 5 days post treatments respectively.

Based on the pharmacokinetic parameters) for groups IV and VI, the C_{max}/MIC ratio of 8.17 and AUC/MIC ratio of 106.41 for difloxacin indicate that difloxacin at oral dose of 30mg/kg b.wt. every 24 hours had a potential clinical efficacy against *K. pneumoniae*.

Keywords. *Efficacy, Difloxacin, Florfenicol, Klebsiella Pneumoniae*

Introduction

*K. pneumoniae*is an opportunistic pathogen of domestic animals and birds (**Sumitha and Sukumar, 2014 and Younis, 2017).** In poultry they are found to be associated with different disease as respiratory infection, septicemia, salpingitis, air sac disease, artheritis, panophthalmitis and intestinal disturbance. Concurrent infection of young poultry with *K. pneumoniae* increased the severity of respiratory disease **(Saif *et al*., 2003).** Weakness, gasping, pump-handled respiration, dyspnoea, mucous discharge, swelling of sinusswes, facial oedema, tracheitis, exudative pneumonia, pleuritis, air sacculitis, pericarditis, sinusitis, drop in egg production and poor egg quality **(Canal *et al.*, 2005)**.

K. pneumoniae is usually multidrug resistant to β-lactams and non β-lactams as fluoroquino-

lone and aminoglycosides **(Gundogan and Avci, 2013)**. Antibiotic sensitivity test of isolated *K. pneumoniae* showed resistant to most commonly used antibiotics except enrofloxacin **(Sumitha and Sukumar, 2014)**. FDA ban the use of enrofloxacin in poultry in the United States in July 2005 **(FDA, 2005)**, in addition, Enrofloxacin is banned by Egyptian Drug Authority (**EDA, 2015**).

It is noted that *K. pneumoniae* resistant to most antibiotics, even antibiotics that have efficacy against *K. pneumoniae* are not authorized to be used in the veterinary practice by the relevant authorities as imipenem (carbapenem class of β -lactam) **(Toshie *et al*., 2013)** which is currently licensed to treat human disease.

Difloxacin is a fluoroquinolone carboxylic acid antimicrobial agent which approved by the responsible authorities and allowed to used in the veterinary field and has high activity against a wide range of gram-positive and gram-negative aerobes and anaerobes, including most species and strains of *Klebsiella, Staphylococcus, E. coli, Enterobacter, Campylobacter, Shigella, Proteus, Pasteurella, Mycoplasma, Rickettsia,* and *Chlamydia* in vitro (**Abd El-Aty *et al*., 2005**). As a member of the fluoroquinolone group, difloxacin acts on bacterial DNA topoisomerases II and IV **(Wolfson and Hooper, 1989**).

Fluoroquinolones are considered to have a concentration-dependent effect. They also have characteristics such as a wide spectrum of bactericidal activity, a large volume of distribution, low plasma-protein binding, and relatively low MICs against target microorganisms **(Brown, 1996).** Principal advantages of fluoroquinolones include good bioavailability, bactericidal activity at low tissue concentrations and good penetration into phagocytic cells **(Giguere *et al.,* 1996)**. They have a large volume of distribution combined with low plasma protein binding, which allows them to reach tissue concentrations often higher than concurrent serum concentrations **(Prescott and Baggot, 1993)**.

Florfenicol, a structural analogue of thiamphenicol, is of great value in veterinary treatment of infectious diseases by inhibiting bacterial protein synthesis at the ribosome **(Cannon *et al* 1990).** The recommended dose is 30 mg/kg bw for 3 days via drinking water **(EMA, 1999)**.

Medication with florfenicol (20mg, 30mg /kg bwt. For five days) greatly reduced the prevalence and severity of clinical sings of colibacillosis **(EL-Banna *et al*., 2007).**

Florfenicol has more antibacterial activity than chloramphenicol and thiamphenicol, including activity against many isolates resistant to chloramphenicol such as *E.coli*, *Proteus vulgaris*, *Salmonella typhimurium* and *Staphylococcus aureus* **(Syriopoulou *et al*., 1981).**

Florfenicol have been shown to be effective against *K. pneumonia* **(Neu and Fu, 1980**). Is the drug still effective against *K. pneumonia* or it has acquired resistance against the drug, This will be one of the objectives of this study by using two different concentrations of the drug.

In poultry farms, the drug combinations are commonly used. These combinations have been studied in chickens and may result either in diminished effects or drug potentiation **(Becker, 2011).** Therefore, the aim of this study was to evaluate the effect of difloxacin and /or florfenicol as a veterinary products in different dosage regimen that can inhibit *K. pneumoniae* to select the appropriate antibiotic and dose regimen.

Materials and Methods

Drug:

Difloxacin:

Difloxacin was obtained as oral solution (10%) from Atco Pharma for Pharmaceutical Industries / Egypt, under trade name "Diflobiotic".

Florfenicol:

Florfenicol was obtained from Pharma Swede / Egypt as oral solution (10%) under trade name "Floricol".

Chickens

sixty three healthy Hubbard broiler chicks

were obtained from a private commercial hatchery, fed on drug free-ration and supplied with water ad-libitum before and during the experiments. Be sure that chicks are free of *K. pneumoniae*.

Bacterial strain
K. pneumoniae was obtained from serology unit, animal health research institute. One ml of *K. pneumonia*e suspension, containing $4x10^3$ CFU.

Experimental design:
Broiler chickens of all groups except the control group were infected by intratracheal injection with 40 µL of the inoculum with a concentration $4x10^3$ CFU/ml of *K. pneumoniae*. Sixty three broiler chickens were divided into 7 equal groups as following :

Group I: Non-infected-non- treated (control negative).
Group II: Experimentally infected with *K. pneumonia* and non-treated (control positive).
Group III: Experimentally infected by *K. pneumonia* and treated with difloxacin (10 mg/ kg b. wt.) once daily, for 7 successive days orally.
Group IV: Experimentally infected by *K. pneumoniae* and treated with difloxacin (30 mg/ kg b. wt.) once daily, for 7 successive days orally.
Group V: Experimentally infected by *K. pneumonia* and treated with florfenicol (20 mg/ kg b. wt.) once daily, for 5 successive days orally.
Group VI: Experimentally infected by *K. pneumonia* and treated with florfenicol (30 mg/ kg b.wt.) once daily, for 5 successive days orally.
Group VII: Experimentally infected by *K. pneumoniae* and treated with florfenicol (15 mg/ kg b. wt.) and difloxacin (15 mg/ kg b. wt.) once daily, for 7 successive days orally.

Antibiotic was administered to groups after appearance of symptoms as mucous discharge and facial edema and the confirmation is done by re-isolation of bacteria from chonnal swabs.

Bacterial re-isolation:
choanal swabs were taken from all groups daily from the next day of drug administration till the slaughter time for re-isolation of *K. pneumoniae*. The swabs were cultivated onto MacConkey's agar and eosin methylene blue-media incubated at 37°C for 24hours. Pure culture of isolates were subjected to biochemical tests according to **Barbara *et al.* (1994)**.
Re-isolation of *K. pneumonia*e from organs
To re-isolate the challenged organism, three birds from each group were sacrificed at days 1,3,5 post cessation of medicines (post treatment). Swabs from liver and lung were inoculated into nutrient broth and platted on to MacConkey agar and eosin methylene blue media incubated at 37°C for 24hr. The organism was identified on the basis of cultural characters and biochemical identification according to **Barbara *et al.* (1994)**. *Klebsiella* organism were counted in liver and lung samples using drop plate technique after 10 fold serial dilution **(Fig. 5)** according to **Barquero-calvo *et al.* (2013).**

Antimicrobial activity of antibiotics:
Antimicrobial activities of antibiotic against *K.pneumoniae* were evaluated by the agar well diffusion method. *K.pneumoniae* strain (0.1 ml of diluted inoculum) was swabbed on the Mueller Hinton plates. Wells (6mm diameter) were cut into the agar and 100 µL of different concentrations of antibacterial agents ranging from 100 to 0.39µg/ml are used. Activity was measured as inhibition zone in millimeters around the well **(Balouiri *et al.,* 2016) (Table 1).**
Minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) of difloxacin and / or florfenicol against *K.pneumoniae*.
MIC was determined using broth macrodilution method. The drugs were diluted in Mueller Hinton broth tubes to give the final concentrations ranged from 100 to 0.195µg/ml. Two fold dilutions of antibiotic was used in Mueller Hinton broth with $5x10^5$ CFU/ml of *K. pneumoniae.* Growth control tube was tested, containing broth without antibiotic. The tubes were incubated at 37°C for 24h.
MIC was the lowest concentration of the antibacterial agent that did not permit any visible growth of bacteria during 24hours of incubation on the basis of turbidity. To determine the

MBC, all the tubes showing no bacterial growth in the MIC test were subcultured. A standard loopful (0.01ml) from each clear tube was subculture on Mueller Hinton plate. The plates are incubated at 35˚C for 18 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum **(Amita *et al*., 2013) (Table 2).**

Evaluation of synergy between difloxacin and florfenicol

The combinations of difloxacin and florfenicol were tested for fractional inhibitory concentration (FIC) determination, are presented in **(Table 3).** Difloxacin at a concentration corresponding to 1/2 MIC was used with florfenicol concentrations ranged from 1/32 MIC to two times of MIC (2×MIC) and vice versa **(Jarrar *et al.*, 2010).** The fractional inhibitory concentration (FIC) value was calculated using the formula **(Kamatou *et al*., 2006).**

FIC index = FIC A + FIC B

Where FIC is the MIC of the combination / MIC of drug alone. The drug interaction classified as:

Synergistic: if the FIC index was <1, so the total effect is stronger than the sum of effects of the individual agents.

Additive: if the FIC index were =1, so the total effect is equal to the sum of effects of the individual agents.

Indifferent: if the FIC index were between 1 and 2, so the total effect is equal to the effect of either individual agent.

Antagonistic: if the FIC index were > 2, so the total *effect* is less than the *sum of* effects of the individual agents.

Pharmacokinetic / pharmacodynamics determination

Pharmacokinetic parameters (C_{max}, t_{max}, $V_{d\,area}$, $t_{0.5\beta}$, AUC_{0-t}) and Pharmacokinetic / pharmacodynamics (T>MIC and C_{max} /MIC) for groups IV and VI were determined. In the first day after inoculation by *K.pneumoniae* and administration of 30 mg/ kg b.wt. of difloxacin and florfenicol, blood samples from the two groups were collected after 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 8 and 12h. Serum was separated by centrifugation at 2000g for 10 min. and stored at -20°C until assay **(Abo-El-Sooud *et al*., 2012).**

Tissue residues of difloxacin and florfenicol

One gram of liver and lung of IV and VI groups at days 1, 3, 5 post treatment were used for determination of drug tissue concentrations according to **San Martin *et al*. (2007)**

Determination of drug concentrations in serum and tissue

Florfenicol and difloxacin concentrations in serum and tissue were measured by the bioassay method using the standard curve of the drugs.

Standard curve of drugs

Standard curve of florfenicol and difloxacin were done using concentrations between 10 to 0.156 µg/ml using *Bacillus subtilis* for florfenicol and *E.coli* for difloxacin as an indicator strain. **(EL-Sayed *et al.*, 1994)**

Determination of The time for which the serum drug levels remain above or equal to MIC (T> MIC %).

T> MIC % value is calculated using the formula (**Turnidge, 1998).**

$$\%T > MIC = ln\left[\frac{D}{Vd(area) \times MIC}\right] \times \left[\frac{t1/2\beta}{ln\,(2)}\right] \times \left[\frac{100}{DI}\right]$$

where:

D: is the proposed dose.

Vd: (area) is the volume of distribution. $t_{1/2\beta}$ is the terminal elimination half-life. DI is the dose interval

Statistical analysis

In this study, the values were expressed as mean ± standard deviation (SD). The significance of the difference between each value presented by various groups was evaluated by one-way analysis of variance (ANOVA) and values with $P < 0.05$ were considered as statistically significant **(Kim, 2014)**. The pharmacokinetic variables were determined using PK Solver: An add-in program for Microsoft Excel, version 2 **(Zhang *et al*., 2010).**

Results and Discussion

The misuse of antibiotic in chickens is often associated with incomplete bacterial eradication, resulting in an insufficient clinical re-

sponse in some cases and the risk of the emerge of antibacterial resistance **(Haritova *et al.*, 2006).** *K. pneumonia*e is a multidrug-resistant bacteria as it is resistant to multiple broad-spectrum antibiotics such as ampicillin and cephalosporins, which were previously helpful in treating these organisms . The mechanism for its resistance is due to the extended beta-lactamases and carbapenemases produced by these bacteria **(Bush and Fisher, 2001)**. Hence, susceptibility testing and clinical efficacy helps to determine which antibiotics would be appropriate in controlling these organisms.
In vitro, Difloxacin was effective in the range of 100 to 0.39 µg/ml against *K.pneumoniae* with zone of inhibition ranged between 30 to 11 mm while florfenicol was effective in the range of 100 to 3.125 µg/ml with zone of inhibition ranged between 25 to 11 mm **(table 1, Fig. 3 and 4)**.

The MIC and MBC values for difloxacin was 0.156 and 0.312 µg/ml while for florfenicol was 3.125 and 6.25 µg/ml against *K. pneumoniae* **(table 2).** The value may be close to that reported by **Aric *et al.*, (2005),** they found that the MIC_{90} of difloxacin against Enterobacteriaceae spp. was ≤ 0.25 µg/mL. In this respect, **Shu-Peng *et al.* (2000)** stated that the MIC_{50} of florfenicol for *K.pneumoniae* was 12.5 µg/ml. The reported MIC breakpoints for difloxacin were ≤ 0.5 µg/mL for susceptible organisms, 1 to 2 µg/mL for intermediate and ≥4 µg/mL for resistant organisms **(NCCLS, 2002)** for Gram negative bacteria while florfenicol breakpoints adapted from Clinical and Laboratory Standards Institute (CLSI) for bovine respiratory disease are 2 µg/mL for susceptible organisms, 4 µg/ml for intermediate and ≥ 8 µg/mL for resistant organisms **(Michael, 2015).**

Antimicrobial resistance seriously threatened animal health. Combination therapy is generally an effective strategy to fight this resistance. In vitro studies, the combination of difloxacin with florfenicol showed an indifferent interaction **(FIC index =1.5**) for *K. pneumonia*e with no occurrences of synergism or antagonism which did not significantly differ from the difloxacin treatment alone.

From the above the results of in-vitro susceptibility testing, *K.pneumoniae* has been shown to be susceptible to difloxacin as the MIC below the susceptible breakpoint.

The laboratory report, provides guidance to clinicians with respect to the potential use of agents in the treatment of patients **(Mouton *et al.*, 2012)**. The use of increasing doses and decreasing treatment durations, in particular, for antibiotic, was also proposed to both avoid treatment failures in infections caused by high-level-resistant bacteria and control the dissemination of resistant strains. (**Guillemot *et al.*, 1998).** Therefore, a clinical trial and using of different dose regimen is conducted to confirm the above results by using two concentrations of difloxacin and florfenicol and both together (difloxacin+florfenicol) to study the extent of their effect on *K. pneumoniae*.

In the present study, all chickens of uninfected untreated group were negative for isolation of *K. pneumoniae* challenge from chaonal swabs and organs while bacteria was isolated from chaonal swabs from Infected-untreated group with the re-isolation rate of 100%. The group treated by difloxacin (30mg/kg b. wt.), the isolation rate was 22.2% while the group treated by difloxacin (10mg /kg b.wt.), florfenicol (20 mg/kg b.wt.), florfenicol (30 mg/kg b.wt.) and both (florfenicol with difloxacin), the re-isolation rate were 77.8%, 88.8%, 88.8%, and 66.7% at 5^{th} day following the first dose respectively **(table 4 and Fig. 6).**
Enumeration of *K. pneumoniae* in the liver of the Infected-untreated group ranged from $3.4x10^3$ to $3.8x10^3$ CFU/gm (log CFU ranged from 3.54±0.01 to 3.57±0.02) while the group treated by difloxacin (30mg/kg b.wt.) significantly differ from the Infected-untreated group and the other groups with CFU/g ranged from $2.6x10^3$ to $8x10^2$CFU/gm (log CFU ranged from 3.39 ± 0.02to 2.96 ± 0.10) during the five days post treatment**) tables 5, 6 and Fig. 1).**

On the other hand, the other treatments are less effective in controlling *K. pneumoniae* infection except the liver of the group **VII** (treated by difloxacin and florfenicol) showed significant decrease in the the bacterial count (log CFU =3.43±0.06) comparing with the control

positive group (3.56±0.01) at 3th day post treatment, but other treatments, no significant changes from the control positive group was recorded.

The lung of the group IV (treated by difloxacin,30mg/kg b.wt.) showed significantly decrease in the bacterial count against the control positive group and other groups, where CFU/gm ranged from $2.8x10^3$to $2.2x10^2$CFU/gm with logCFU ranged from 3.43±0.017 to 3.35± 0.02 while the control positive group was $3.6x10^3$ to $4.0x10^3$ CFU/gm with logCFU ranged from 3.57±0.017 to 3.59±0.01 throughout the experiment **(tables 7, 8 and Fig. 2).**

The Group VII (treated by difloxacin and florfenicol) revealed significant decrease in the bacterial count in the lung (log CFU = 3.49±0.01 and 3.48±0.01) compared with the control positive group (log CFU = 3.58±0.011 and 3.59±0.01) and with group III (3.55±0.02 and 3.54±0.01) at 3thand 5thday post treatment respectively.

This result is due to the concentration of difloxacin in group VII (15mg/kg b.wt) is more than group III (10mg/kg b.wt) and the relation between difloxacin and florfenicol showed an indifferent interaction.

The group treated with difloxacin (10mg/kg b.wt, Group III) showed significant decrease in the lung bacterial count (log CFU = 3.54±0.011) compared with the control positive group (log CFU = 3.59±0.01) and with group VI (log CFU = 3.57±0.017) at 5th day after treatment.

Based on above, the killing activity of difloxacin 30mg/kg b.wt was found to be greater against *k.pneumoniae* than other treatments followed by the combination of difloxacin with florfenicol then difloxacin 10mg/kg b.wt.
The findings of the present study are supported by the previous reports, whereby **Aric *et al.* (2005**) found that difloxacin appears to be safe, adequately absorbed and may be useful in the treatment of *K. Pneumoniae* infections in adult horses. In addition, Klebsiella spp. was found to be susceptible to difloxacin in vitro by **EMA, (2008**).

Furthermore, other fluoroquinolones may showed activity against *K. pneumonia* infection. **Antoine *et al.,* (2016)** reported that *K. pneumoniae* were susceptible to levofloxacin and moxifloxacin in vitro. In addition, ciprofloxacin has bactericidal effect against growing *K. pneumoniae* and non-growing *K. pneumonia* (late infection model), but the extent of killing was higher on growing bacteria and by increasing the dosage of ciprofloxacin from 40 to 200 mg/kg, can compensate the lower killing in low-level ciprofloxacin-resistant *K. pneumoniae*. **(Kurt and Helga, 2002)**. Moreover, Ofloxacin has shown activity against *K. pneumoniae* **(Abdullah *et al.,* 2013)**. After single oral administration of enrofloxacin to the affected rabbits with *K.pneumoniae* at a dose of 10 mg/kg b.wt. for five days, the mortality rate was reduced gradually by the 3rd day of initiation of treatment **(Sumitha and Sukumar, 2014).** Sitafloxacin and clinafloxacin displays antibacterial activity against ciprofloxacin-resistant *K.pneumoniae* (**Brisse *et al.,* 2000**).

In this respect, **Ghanem *et al.* (2015**) found that *K.pneumoniae* was sensitive to florfenicol, highly sensitive to tulathromycin and moderately sensitive to amoxicillin. Similar results were reported by **Aslan *et al.* (2002),** they stated that florfenicol has a high bacteriological and clinical efficacy in the treatment of calf respiratory tract diseases due to mixed infection including *K. pneumoniae* (20%). Florfenicol has a broad spectrum of antibacterial activity versus clinical or subclinical bovine mastitis caused by Klebsiella species **(Wilson *et al.,* 1996**).

On the other hand , some recent studies highlight the emergence of multidrug resistant *K. pneumoniae* strains including the resistance to florfenicol and fluoroquinolones. **Junwan *et al.*, (2018**) demonstrated that 20.42% of the *clinical K. pneumonia*e isolates were resistant to florfenicol, but only 7.01% carried the floR gene and 86.96% of the floR-positive strains demonstrated high resistance to florfenicol with MICs ≥ 512 μg/mL. Resistance to fluoroquinolones have been shown to be due primarily to alterations in gyrA, which encodes DNA gyrase, a type II topoiso-

merase **(Yingmei *et al.,* 2008)** .

After repeated oral administration of drugs (**table 9),** the tissue concentration of difloxacin in slaughtered chickens at a dose of 30 mg/ kg. b.wt once daily for 7 days (group **IV**) were 0.58±0.03 and 0.47±0.06 µg/gm in the lung and liver (the target tissues) at the 5th day post treatment, which were above the MIC of difloxacin against *K.pneumoniae* while florfenicolat a dose of 30mg/kg. b.wt once daily for 5 days (group **VI**), the residues in liver and lung were 0.8±0.10 and 0.46±0.06 µg/gm at the 5th day post treatment respectively and these values below the MIC of florfenicol.

These results were supported by a previous results reported by (**Samah *et al*., 2012**) where they found that oral dose of florfenicol (30 mg/ kg b.wt. for 5 days), liver concentration was 0.48 µg/gm at 5th day post last dose and tissue disposition of florfenicol were persisted up to 7 days.

Arturo *et al*. (2011) reported that a withdrawal time of 5 days for difloxacin was necessary to ensure that the residues after multiple oral dose (10mg difloxacin/kg b.wt., daily for 5 days) were less than the maximal residue limits.
Combined use of pharmacokinetic (PK) and pharmacodynamics (PD) helps us to optimize effective use of an antimicrobial agent. Integrated use of PK and PD data provides a rational basis to understand the impact of various dosage regimens on the time course of pharmacologic responses. It provides information on the effective dose and duration of therapy of a specific agent against a specific pathogen. Pharmacokinetic-pharmacodynamic studies support the possibility that dose patterns may affect the selection of resistance and bacterial eradication (**Drusano *et al.,* 1993).**

The pharmacokinetic study of florfenicol and difloxacin was studied for groups IV and VI **(tables 10, 11).** Florfenicol acts as a time-dependent drug and the most important pharmacodynamics /pharmacokinetic parameter for this type of drug is the length of the time during which drug remains above the MIC_{90} value. It is generally recommended that T>MIC should be at least 50% of the dosage interval to ensure an optimal bactericidal effect (**Toutain and Lees, 2004).**

The experimental data shows that C_{max}, AUC and V_d of florfenical were 5.24±0.43µg/ml, 29.8±2.8 µg/ml.h and 5.89±0.51 (mg)/(µg/ml), while MIC against *K.pneumoniae* was 3.125 µg/ml. Florfenicolat dose of 30 mg/kg b.wt. orally at 12 h or 24 h interval, the T>MIC were 13.63% and 27.20% respectively which is not enough to remain the plasma drug levels above or equal to the minimal inhibitory concentration (MIC) value. This dosage regimen predicted an ineffective treatment against *K.pneumoniae*.

The obtained findings were in agreement with results previously reported in broiler chickens **(Shen *et al.,* 2003),** they found that C_{max} and AUC of florfenicol were 5.82 µg/ml and 27.59 mg. h/L after oral dose of 30mg/kg b.wt. respectively.

In contrast, Fluoroquinolonesappearsasa concentration dependant antibacterial agents **(Abo -EL-Sooud *et al*., 2017)**. The AUC/MIC ratio is the most important factor in predicting efficacy, with the rate of clinical success being greater than 80%, when this ratio is higher than 100–125 **(Lode *et al*., 1998).** A second predictor of efficacy for concentration dependent antibiotic is the ratio C_{max}/MIC, considering that values above 8–10 would lead to better clinical results and to avoidance of bacterial resistance emergence **(Walker, 2000).** Following oral administration of difloxacin, 30mg/kg b.wt., the C_{max}, AUC_{0-t} and V_d area were 2.55±0.23 µg/ml, 16.6±0.95 µg/ml.h and 10.77±0.46 (mg)/ (µg/ml). The C_{max} /MIC ratio was 8.17 and AUC/MIC ratio was 106.41 .

The obtained results were in close to that reported by **Abo El-Elaa *et al*. (2014)**, they reported that C_{max} and AUC_{0-t} of difloxacin were 1.34µg/ml and 12.16µg/ml.h after oral administration of 10mg/kg b.wt. respectively .

The findings of the present study indicate that difloxacin at dose 30mg/kg b.wt. has more activity than other treatments against *K. pneumoniae* and a dose of 30 mg/kg b.wt. of diflox-

acin given orally every 24 h in chickens can maintain effective serum concentrations against *K.pneumoniae*.

Conclusion

The findings of the present study indicate that difloxacin therapy at 30 mg/kg b.wt. every 24 h for 7 days has been shown to be highly effective in the treatment of *K. pneumonia* while florfenicol alone is not effective against *K. pneumoniae*. The combination of difloxacin with florfenicol showed an indifferent interaction against for *K. pneumoniae.*

Table (1). Antibacterial activity (zone of inhibition) of difloxacin and florfenicol against *K. pneumoniae* by agar well diffusion method

Drug conc. (µg/ml)	Diameter of zone of inhibition (mm)	
	Difloxacin	Florfenicol
100	30	25
50	27	22
25	25	18
12.5	22	15
6.25	20	13
3.125	17	11
1.563	15	ND
0.781	13	ND
0.390	11	ND
0.195	ND	ND

ND : not detected

Table (2). Minimum inhibitory concentration (MIC) and minimum bactericidal conc. (MBC) of difloxacin and florfenicol against *K. pneumoniae*

Drug	MIC (µg/ml)	MBC (µg/ml)
difloxacin	0.156	0.312
florfenicol	3.125	6.25

Table (3). Fractional inhibitory concentration (FIC) index

Drug	MIC alone (µg/ ml)	MIC in combination (µg/ ml)	FIC
Difloxacin	0.156	0.156	1.0
Florfenicol	3.125	1.562	0.5
FIC index	1.5 (indifferent)		

Table (4). Re-isolation of *K.pneumoniae* in choanal swab of broiler chickens experimentally challenged by *k. pneumoniae* (No. of positive samples /No. of broilers examined, percentage in parentheses) in different groups (n=9).

Groups	Number of Examined chickens	Number and percentage of positive chickens with *k. pneumoniae*						
		1st day from the first dose	2 nd day from the first dose	3rd day from the first dose	4th day from the first dose	5th day from the first dose	6th day from the first dose	7th day from the first dose
Control negative (Group I)	9	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)
Control positive (Group II)	9	9/9 (100%)	9/9 (100%)	9/9 (100%)	9/9 (100%)	9/9 (100%)	9/9 (100%)	9/9 (100%)
Difloxacin (10 mg) (Group III)	9	9/9 (100%)	9/9 (100%)	8/9 (88.8%)	8/9 (88.8)	7/9 (77.8%)	7/9 (77.8%)	6/9 (66.7%)
Difloxacin (30mg) (Group IV)	9	6/9 (66.7%)	6/9 (66.7%)	5/9 (55.6%)	4/9 (44.4%)	2/9 (22.2%)	2/9 (22.2%)	1/9 (11.1%)
Florfenicol (20mg) (Group V)	9	9/9 (100%)	9/9 (100%)	9/9 (100%)	9/9 (100%)	8/9 (88.8%)	NR	NR
Florfenicol (30mg), (Group VI)	9	9/9 (100%)	9/9 (100%)	9/9 (100%)	8/9 (88.8)	8/9 (88.8%)	NR	NR
Difloxacin + florfenicol (Group VII)	9	9/9 (100%)	9/9 (100%)	8/9 (88.8%)	8/9 (88.8%)	6/9 (66.7%)	6/9 (66.7%)	5/9 (55.56%)

NR : not required where florfenicol was administered for 5 days

Table (5). Enumeration of *K.pneumoniae* in liver of experimental broiler chicken, CFU/g (n=3).

Time	bacteria	Control negative	Control Positive	difl. 10mg	difl. 30mg	Flor. 20mg	Flor. 30mg	dif.+flor,
1st day post treatment	**No. of colonies**	0	$3.4x10^3$	$3.6x10^3$	$2.4x10^3$	$3.6x10^3$	$3.6x10^3$	$3.2x10^3$
		0	$3.6 x 10^3$	$3.6x10^3$	$2.4x10^3$	$3.4x10^3$	$3.6x10^3$	$3.2x10^3$
		0	$3.6x10^3$	$3.2x10^3$	$2.6x10^3$	$3.4x10^3$	$3.4x10^3$	$3.4x10^3$
3nd day post treatment	**No. of colonies**	0	$3.8x10^3$	$3.6x10^3$	$1.8x10^3$	$3.8x10^3$	$3.2x10^3$	$3.2x10^3$
		0	$3.8x10^3$	$3.2x10^3$	$1.8x10^3$	$3.2x10^3$	$3.6x10^3$	$2.6x10^3$
		0	$3.6x10^3$	$2.6x10^3$	$1.6x10^3$	$3.2x10^3$	$3.2x10^3$	2.4x10
5th day post treatment	**No. of colonies**	0	$3.6 x10^3$	$3.6x10^3$	$1.2x10^3$	$3.6x10^3$	$3.8 x10^3$	$2.6x10^3$
		0	$3.8 x10^3$	$2.8x10^3$	$8x10^2$	$3.6x10^3$	$2.8x10^3$	$2.4x10^3$
		0	$3.8 x10^3$	$3.0x10^3$	$8x10^2$	$3.0x10^3$	$3.2x10^3$	$2.6x10^3$

Dil: dilfloxacin
Flor: florfenicol

Table (6). Log CFU of *K. pneumoniae* in liver of experimental broiler chicken (Mean ± S.D.) (n=3).

Time	Control positive	difl. 10mg	difl. 30mg	Flor. 20mg	Flor. 30mg	dif.+flor
1st day post treatment	3.54 ± 0.011[b]	3.52 ± 0.017[b]	3.39 ±0.017[a]	3.53 ± 0.012[b]	3.54 ± 0.012[b]	3.51 ± 0.017[b]
3rd day posttreatment	3.56±0.01[b]	3.48± 0.07[b]	3.24 ± 0.034[a]	3.52± 0.04[b]	3.51±0.02[b]	3.43±0.06[a b]
5th day post treatment	3.57 ± 0.02[b]	3.49 ± 0.05[b]	2.96 ± 0.10[a]	3.52 ± 0.04[b]	3.51 ± 0.06[b]	3.40 ± 0.02[b]

[a] significantly different from the control group at $p< 0.05$
[b] significantly different from difloxacin 30 group at $p< 0.05$

Table (7). Enumeration of *K.pneumoniae* in lung of experimental chicken (CFU/g) (n=3).

Time	bacteria	Control negative	Control positive	difl. 10mg	difl. 30mg	Flor. 20mg	Flor. 30mg	dif.+flor
1st day post treatment	No. of colonies	0	$3.8x10^3$	$3.6x10^3$	$2.8x10^3$	$3.8x10^3$	$3.6x10^3$	$3.6x10^3$
		0	$3.8x10^3$	$3.4x10^3$	$2.6x10^3$	$3.6x10^3$	$3.6x10^3$	$3.4x10^3$
		0	$3.6x10^3$	$3.8x10^3$	$2.8x10^3$	$3.8x10^3$	$3.8x10^3$	$3.4x10^3$
3rd day post treatment	No. of colonies	0	$3.8x10^3$	$3.4x10^3$	$2.6x10^3$	$3.8x10^3$	$3.6 x10^3$	$3.0x10^3$
		0	$3.8x10^3$	$3.8x10^3$	$2.4x10^3$	$3.8x10^3$	$3.6 x10^3$	$3.2x10^3$
		0	$4.0x10^3$	$3.6x10^3$	$2.4x10^3$	$3.6x10^3$	$3.8 x10^3$	$3.0x10^3$
5th day post treatment	No. of colonies	0	$4.0x10^3$	$3.6x10^3$	$2.2x10^2$	$3.8x10^3$	$3.8x10^3$	$3.0x10^3$
		0	$4.0x10^3$	$3.4x10^3$	$2.4x10^2$	$3.6x10^3$	$3.8x10^3$	$3.0x10^3$
		0	$3.8x10^3$	$3.6x10^3$	$2.2x10^2$	$3.8x10^3$	$3.6x10^3$	$3.2x10^3$

Table (8). Log CFU of *K. pneumoniae* in lung of experimental chicken (Mean ± S.D.) (n=3).

Time	Control positive	difl. 10mg	difl. 30mg	Flor. 20mg	Flor. 30mg	dif.+flor
1st day post treatment	3.57 ± 0.017[b]	3.55 ± 0.025[b]	3.43 ± 0.017[ac]	3.57 ± 0.018[b]	3.56 ± 0.017[b]	3.53 ± 0.011[b]
3rd day post treatment	3.58±0.011[b]	3.55± 0.02[b]	3.39 ± 0.01[ac]	3.56± 0.01[b]	3.56 ± 0.01[b]	3.49 ±0.01[abc]
5th day post treatment	3.59 ± 0.01[bc]	3.54 ± 0.011[ab]	3.35 ± 0.02[ac]	3.57 ± 0.017[b]	3.57 ± 0.017[bc]	3.48 ± 0.01[abc]

[a] significantly different from the control group at $p< 0.05$
[b] significantly different from difloxacin 30 group at $p< 0.05$
[c] significantly different from difloxacin 10 group at $p< 0.05$

Table (9). Mean ± S.D. of difloxacin and florfenicol residues (µg/g) after oral administration once daily for five consecutive days in Group IV and Group VI (n=3).

Treatment regimen	Drug and Dose	1st day Post treatment		3rd day Post treatment		5th day Post treatment	
		lung	liver	lung	liver	lung	Liver
Group IV	**Difloxacin (30mg/Kg b.wt.)**	1.90±0.10	1.53±0.06	1.13±0.05	0.92±0.02	0.58 ±0.03	0.47±0.06
Group VI	**florfenicol (30mg/Kg b.wt),**	2.13± 0.06	1.1±0.26	1.46±0.11	0.76±0.05	0.80±0.10	0.46±0.06

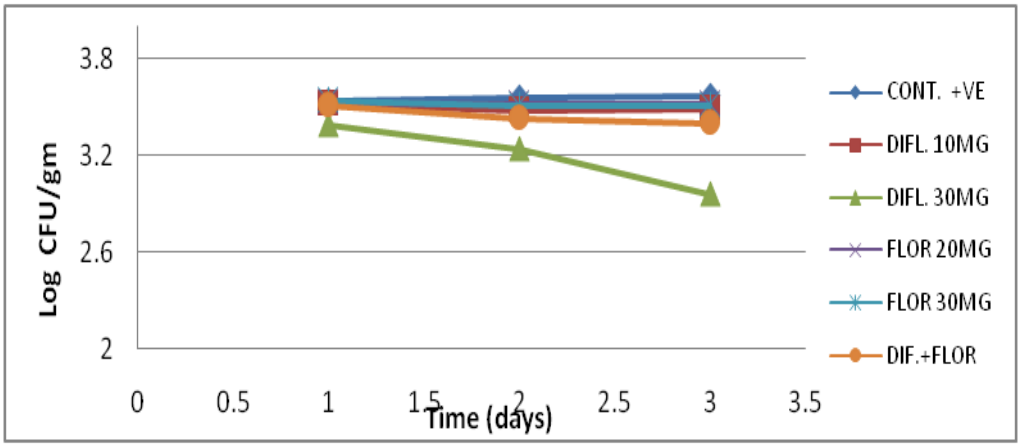
Table (10). Pharmacokinetic parameters of difloxacin and florfenicol in groups IV and VI (Mean ± S.D.) (n=9).

Drug	DOSE	C_{max}	T_{max}	AUC $_{0-t}$	V_d	$t_{0.5\beta}$
Difloxacin	30 mg/kg b.wt.	2.55±0.23 µg/ml	1.70± 0.09 h	16.6±0.95 µg/ml.h	10.77± 0.46 (mg)/(µg/ml)	4.84 ± 0.41h
Florfenicol	30 mg/kg b.wt.	5.24±0.43 µg/ml	1.60 ± 0.07 h	29.8 ± 2.8 µg/ml.h	5.89 ± 0.51 (mg)/(µg/ml)	4.62 ± 0.33 h

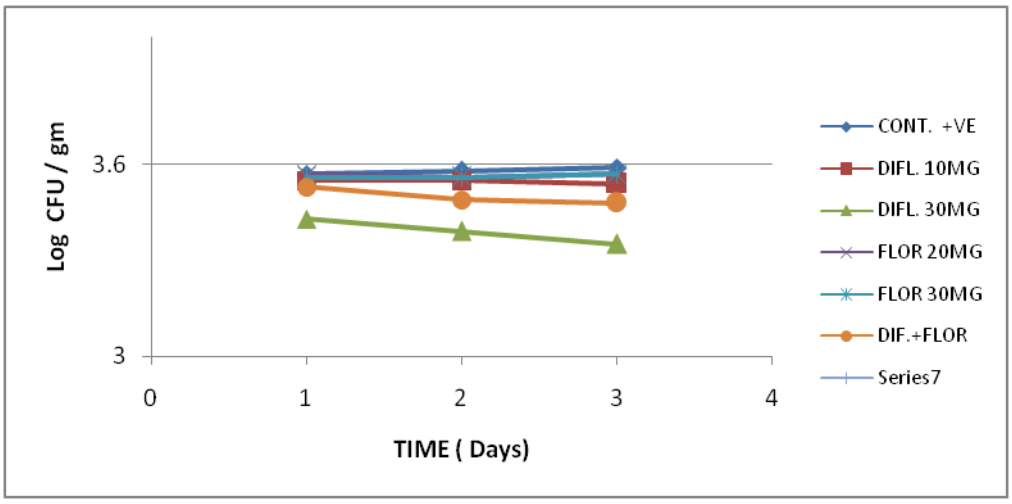
AUC_{0-t} = areas under the concentration time curves;
$t_{0.5\beta}$ = the half-life of the b phase;
C_{max} = maximum concentration;
t_{max} = time to maximum concentration
V_d = volume of distribution

Table (11). Pharmacokinetic / pharmacodynamic integration of difloxacin in groups IV and VI .

Parameters	Difloxacin	florfenicol
T>MIC % Dose interval (24h)	64.04%	13.63%
T>MIC% Dose interval (12h)	128.08%	27.20%
C_{max}/MIC ratio	8.17	1.68
AUC /MIC ratio	106.41	9.54



(Figure 1). Antibacterial activity of different treatments against *K. pneumoniae* in liver of broiler chick-ens



(Figure 2). Antibacterial activity of different treatments against *K. pneumoniae* in lung of broiler chickens

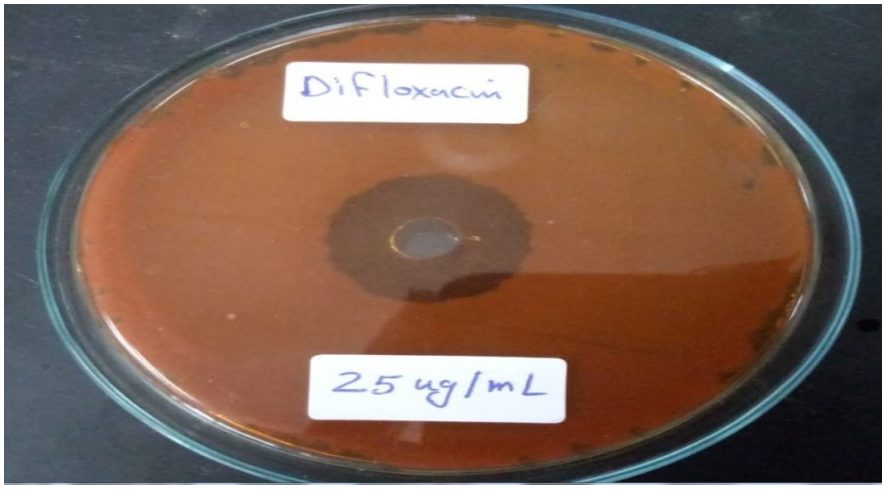


Figure (3). In vitro, Zone of inhibition of difloxacin against *K. pneumonia*e

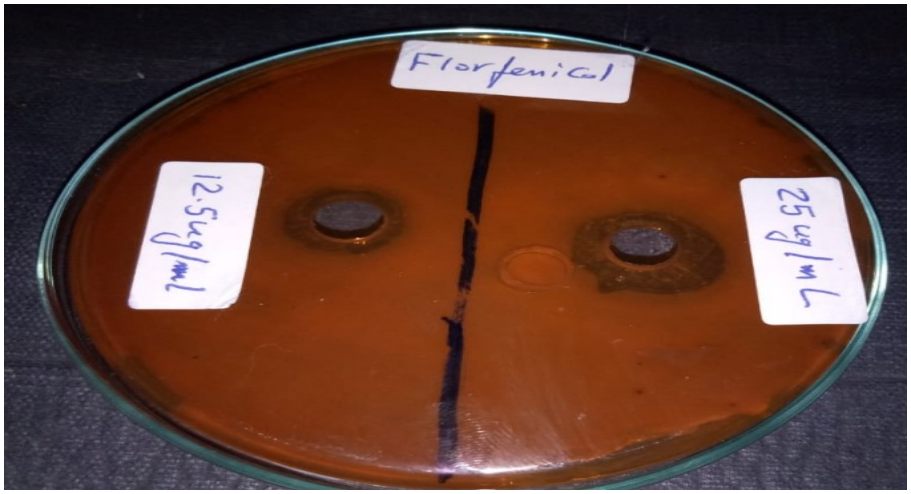


Figure (4). In vitro , Zone of inhibition of Florfenicol against *K. pneumoniae*

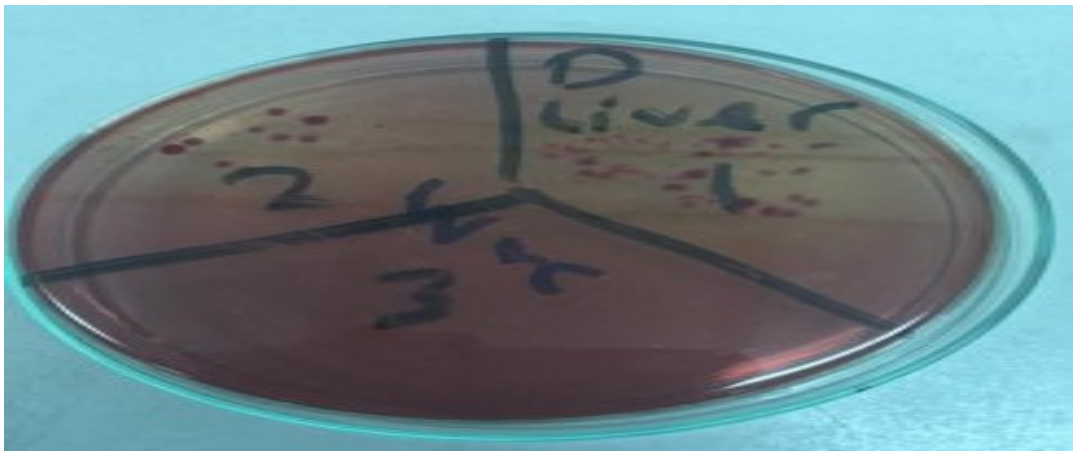


Figure (5). Drop plate technique for of bacterial count of *K. pneumonia*e from liver of broiler chickens on MacConkey's agar media

Figure (6). Bacterial isolation of *K. pneumonia*e in choanal swab of broiler chickens on MacConkey's agar media

References

Abd El-Aty, A.M.; Goudah, A. and Ismail, M. (2005). Disposition kinetics of difloxacin in rabbit after intravenous and intramuscular injection of dicural. Vet. Res. Commun.; 29: 297–304.

Abdullah, F.E.; Mushtaq, A.; Irshad, M.; Rauf, H.; Afzal, N. and Rasheed, A. (2013). Current efficacy of antibiotics against *Klebsiella* isolates from urine samples, a multicentric experience in Karachi. Pak. J. Pharm. Sci., 26(1): 11-5.

Abo El-Ela, F.I.; Radi, A.M.; El-Banna, H.A.; El-Gendy, A.A.M. and Tohamy, M.A. (2014). Pharmacokinetics of difloxacin in healthy and E.coli-infected broiler chickens. British Poultry Science, 55, 6, 830–836.

Abo-EL-Sooud, K.; Ahmed, M.S.; Goudah, A. and Sarah, F.S. (2017). Comparative serum concentrations and pharmacokinetics of levofloxacin and danofloxacin in broiler chickens**.** Wulfenia Journal, 24(1), 225-237.

Abo-EL-Sooud, K.; Afifi. N.A. and Abd-El-Aty, A.M. (2012). Pharmacokinetics and bioavailability of tulathromycin following intravenous, intramuscular and subcutaneous administrations in healthy rabbits. Vet. World, 5(7), 424-428.

Amita, M.; Shashank, K. and Abhay, K. (2013). Scientific validation of the medicinal efficacy of Tinospora cordifolia. Scientific World Journal, P, 1-8.

Antoine, G.; Frédéric, S.; Magali, K. and François, J. (2016). Comparative Activity of Ciprofloxacin, Levofloxacin and Moxifloxacin against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and Stenotrophomonasmaltophilia assessed by minimum inhibitory concentrations and time-kill studies. PLoSOne., 11(6).

Aric, R.A.; Gregory, R.H.; Murray, P.B.; Ronald, G. and Kelly, M. (2005). Pharmacokinetics of difloxacin and its concentration in body fluids and endometrial tissues of mares after repeated intragastric administration. The Canadian Journal of Veterinary Research , 69, 229–235.

Arturo, A.; Frank, S.; Maria, A.M. and Víctor, C. (2011). Plasma disposition and tissue depletion of difloxacin and its metabolite sarafloxacin in the food producing animals, chickens for fattening. Food and chemical toxicology journal 49(2), 441-449.

Aslan, V.; Maden, M.; Erganis, O.; Birdane, F.M. and Corlu, M. (2002). Clinical efficacy of florfenicol in the treatment of calf respiratory tract infections. Veterinary Quarterly, 24(1), 35-39.

Balouiri, M.; Sadiki, M. and Ibnsouda, S.K. (2016). Methods for in-vitro evaluating antimicrobial activity, A review. Journal of Pharmaceutical Analysis 6(2) , 71-79.

Barbara, J.; John, F.K.; Alices, W.; Thomas, F.S. and Richard, C.T. (1994). Clinical and pathogenic microbiology, Second edition, Mosby, London.

Barquero-Calvo, E.; Chaves-Olarte, E.; Chacon, Diaz C. and Moreno, E. (2013). Bacterial counts in spleen. Bio-protocol, 3, Iss 21, 05, 1-6.

Becker, D.E. (2011). Adverse drug interactions. Anesthesia Progr., 58: 31-41.

Brisse, S.; Milatovic, D.; Fluit, A.C.; Verhoef, J. and Schmitz, F.J. (2000). Epidemiology of quinolone resistance of *Klebsiella pneumoniae* and *Klebsiella oxytoca* in Europe. Eur. J. Clin. Microbiol. Infect. Dis. , 19(1): 64-8.

Brown S.A. (1996). Fluorquinolones in animal health. J. Vet. Pharmacol. Ther., 19: 1–14.

Bush, K. and Fisher, J. (2001). Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gramnegative bacteria. Annu. Rev., 65: 455–478.

Canal, C.W.; Leao, J.A.; Rocha, S.L.; Macagnan, M.; Lima-Rose, C.A.; Oliveira, S.D. and Back, A. (2005). Isolation and characterization of *Ornithobacterium rhinotracheale* from chickens in Brazil. Research in Veterinary Science ,78, 225-230.

Cannon, M.; Harford, S. and Davies, J. (1990). A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated analogs. Journal of Antimicrobial Chemotherapy 26, 307 – 317.

Drusano, G.L.; Johnson, D.E.; Rosen, M. and Standiford H.C. (1993). Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of Pseudomonas sepsis. Antimicrob. Agents Chemother. 37: 483-490.

EDA (Egyptian Drug Authority) (2015). Non registration of Enrofloxacin . Ministry Of Health, Central Administration of Pharmaceutical Affairs, Egypt, july, 1-10.

EL-Banna, H.A.; Zaghlol, A.H. and Rehab, M. (2007). Efficacy and tissue residue depletion of florfenicol (water soluble formulation) in healthy and E. coli infected broiler chickens. Research Journal of Biological Sciences, 2 (3): 319-325.

El-Sayed, M.G.A.; Abd El-Aziz, M.I.; El-Komy, A.A. and El-Din, H.S. (1994). Effect of aflatoxin B1 on pharmacokinetic pattern of spectinomycin in chicken. Beni-swef Veterinary Medical Researshes, Egypt, 4: 364-379.

EMA (European Medicines Agency), (2008). Dicural (Difloxacin). EMA/573644, EMEA/V/C/000031.

EMA (European Medicines Agency), (1999). Committee for Medicinal Products for Veterinary Use flofenicol (extension to chickens), summary report 3. March. EMA/MRL /589/99.

FDA (Food and Drug Administration) (2005). Withdrawal of approval of the new animal drug application for enrofloxacin in poultry. Accessed 25, July .

Giguere, S.; Sweeney, R.W. and Belanger, M. (1996). Pharmacokinetics of enrofloxacin in adult horses and concentration of the drug in serum, body fluids, and endometrial tissues after repeated intragastrically administered doses. American Journal of Veterinary Research, 57, 1025– 1030.

Ghanem, M.M.; Yousif, H.M.; Helal, M.A.Y.; Abd El-Raof, Y.M. and El-Attar, H.M. (2015). Comparative therapeutic effect of three types of antibiotics on pneumonia associated with *Klebsiella pneumoni-*

ae in boer goats. Benha Veterinary Medical Journal, 29, (1), 74-84 .

Guillemot, D.; Carbon, C.; Balkau, B.; Geslin, P.; Lecoeur, H.; Vauzelle-Kervroedan, F.; Bouvenot, G. and Eschwege, E. (1998). Low dosage and long treatment duration of beta-lactam: risk factors for carriage of penicillin-resistant Streptococcus pneumoniae. JAMA 279: 365-370.

Gundogan, N. and Avci, E. (2013). Prevalence and antibiotic resistance of extended beta-latamase (ESBL) producing *Escherichia Coli* and *Klebsiella* species isolated from foods of animal origin in Turkey. African Journal of Microbiology Research,7(31), 4059 -4064.

Haritova, A.M.; Rusenova, N.V.; Parvanov, P.R.; Lashev, L.D. and Fink-Gremmels, J. (2006). Pharmacokinetic–pharmacodynamic modelling of danofloxacin in Turkeys. Veterinary Research Communications, 30(7), 775–789.

Jarrar, N.; Adwan, K.M. and Abu-Hijleh, A. (2010). Antibacterial activity of Rosmarinus officinalis L. alone and in combination with cefuroxime against methicillin--resistant *Staphylococcus aureus*. Asian Pacific Journal of Tropical Medicine 3(2): 121-123.

Junwan, L.; Jinfang, Z.; Lei, X.; Yabo, L.; Pingping, L.; Tingyuan, Z.; Cong, C.; Shunfei, L.; Teng, X.; Huiguang, Y.; Kewei, L.; Wu, Z.; Peizhen, L.; Liyan, N. and Qiyu, B. (2018). Spread of the florfenicol resistance *floR* gene among clinical *Klebsiella pneumoniae* isolates in China. Antimicrob. Resist. Infect. Cont., 7: 127.

Kamatou, G.P.P.; Viljoen, A.M.; Van-Vuuren, S.F. and Van-Zyl, R.L. (2006). In vitro evidence of antimicrobial synergy between Salvia chamelaeagnea and Leonotis leonurus. South African Journal of Boteny, 72(4), 634-636.

Kim, H.Y. (2014). Analysis of variance (anova) companing means of more than two groups. Restorative Dentistry and Endodontics, ISSN 2234-7658.

Kurt, F. and Helga, S. (2002). Significance of low-level resistance to ciprofloxacin in *Klebsiella pneumoniae* and the effect of increased dosage of ciprofloxacin in vivo using the rat granuloma pouch model. Journal of Antimicrobial Chemotherapy 50, 421–424

Lode, H.; Borner, K. and Koeppe, P. (1998). Pharmacodynamics of fluoroquinolones. Clinical Infectious Diseases. 27 (1), 33–39.

Michael, D. (2015). Antimicrobial Notes, Pharm II, Kansas State University, 1-119.

Mouton, J.W.; Brown, D.F.J.; Apfalter, P.; Canton, R.; Giske, C.G.; Ivanova, M.; MacGowan, A.P.; Rodloff, A.; Soussy, C.J.; Steinbakk, M. and Kahlmeter, G. (2012). The role of pharmacokinetics pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. Clinical Microbiology and Infection, European Society of Clinical Microbiology and Infectious Diseases, CMI, 18, E37–E45

Neu, H.C. and Fu, K.P. (1980). In vitro activity of chloramphenicol and thiamphenicol analogs. Antimicrobial Agents and Chemotherapy 18, 311– 316.

NCCLS (National Committee for Clinical Laboratory Standards) (2002). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standards; second edition. NCCLS document M31-A2 .

Prescott, J.F. and Baggot, J.D. (1993). Fluoroquinolonesin Antimicrobial Therapy in Veterinary Medicine, 2nd edn, Iowa State University Press, Ames, IA, USA. P: 252–262.

Saif, Y.M.; Barnes, H.J.; Glisson, J.R.; Fadly, A.M.; McDougald, L.R. and Swayne D.E. (2003). Diseases of Poultry, 11th edn. Iowa State University Press, Blackwell Publishing Company, London, United Kingdom pp. 849.

Samah, K.; Eslam, H. and Ola, H. (2012). Residue withdrawal of florfenicol from the serum and edible tissues of broiler chickens . Journal of American Science, 8(12), 514-524.

San Martín, B.; Cornejo, J.; Iragüen, D.; Hidalgo, H. and Anadón, A. (2007). Depletion Study of enrofloxacin and its metabolite ciprofloxacin in edible tissues and feathers of white leghorn hens by liquid chromatography coupled with tandem mass spectrometry .J. Food Prot., 70 (8): 1952-1957.

Shen, J.; Hu, D.; Wu, X. and Coats, J.R. (2003). Bioavailability and pharmacokinetics of florfenicol in broiler chickens. J. vet. Pharmacol. Therap. 26, 337–341.

Shu-Peng, H.; Tain-Yao, H.S.U.; Ming-Hui, C. and Way-Shyan, W. (2000). Antibacterial effect of chloramphenicol, thiamphenicol and florfenicol against aquatic animal bacteria. J. Vet. Med. Sci. 62(5), 479–485.

Sumitha, P. and Sukumar, K. (2014). An outbreak of *Klebsiella pneumoniae* infection in a rabbit farm. Int. J. Curr. Microbiol. App. Sci ., 3(11) 789-790.

Syriopoulou, V.P.; Harding, A.L.; Golgmann, D.A. and Smith, L. (1981). In-vitro antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. Antimicrobial Agents and Chemotherapy 19, 294–297.

Toshie, H.; Shizuo, K.; Ryuichi, K.; Seiya, K.; Norifumi, S.; Makoto, O.; Michiya, Y.; Hiroki, O. and Motoyuki, S. (2013). Meropenem Resistance in Imipenem-Susceptible Meropenem-Resistant *Klebsiella pneumoniae* isolates not detected by Rapid Automated Testing Systems. J. Clin. Microbiol., 51(8): 2735–2738.

Toutain, P.L. and Lees, P. (2004). Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. J. Vet. Pharmacol. Ther., 27, 467-477.

Turnidge, J.D. (1998). The Pharmacodynamics of Beta-Lactams. Clic. Infect. Dis. 27, 10-22.

Walker, R.D. (2000). The use of fluoroquinolones for companion animal antimicrobial therapy. Australian Veterinary Journal. 78 (2), 84–90.

Wilson, D.J.; Sears, P.M.; Gonzalez, R.N.; Smith, B.S.; Schulte, H.F.; Bennett, G.J.; Das, H.H. and Johnson, C.K. (1996). Efficacy of florfenicol for treatment of clinical and subclinical bovine mastitis. Am. J. Vet. Res., 57(4), 526-528.

Wolfson, J.S. and Hooper, D.C. (1989). Fluoroquinolone antimicrobial agents. Clin. Microbiol. Rev., 2: 378–424.

Yingmei, F.; Lishuang, G.; Yan, X.; Wenli, Z.; Jiaao, G.; Jianfeng, X.; Xiaobei, C.; Yuehui, Z.; Jiayu, M.; Xinghan, L. and Fengmin, Z. (2008). Alteration of Gyr A amino acid required for ciprofloxacin resistance in *Klebsiella pneumoniae* isolates in China. Antimicrobial Agents and Chemotherapy, 52, 8 , 2980–2983.

Younis, A.I. (2017). Using of conventional and recent techniques for identification of Klebsiella in diseased broilers. MVSc thesis, Bacteriology, Mycology and immunology, Faculty of Veterinary Medicine, Kafr el-sheikh University.

Zhang, Y.; Huo, M.; Zhou, J. and Xie, S. (2010). PK Solver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Computer methods and programs in biomedicine, 99(3): 306 -314.