Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Investigation of kinetic and tissue residues of cefquinome after its intramuscular administration in experimentally infected rabbits with *Klebsiella pneumoniae*

Maha S. Abd Elhafeez and Mai A. Fadel

Department of chemistry, toxicology and feed deficiency, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Dokki, Giza, Egypt

Article History
Received in 1/11/2020
Received in revised from 23/11/2020
Accepted in 20/12/2020

Keywords:
Kinetic
Residues
Cefquinome
K. pneumoniae
Rabbits

ABSTRACT

The kinetic and tissue distribution of cefquinome were studied after its intramuscular administration at a dose of 2 mg/kg b.wt in healthy and experimentally infected rabbits with *klebsiella pneumoniae.* The cefquinome serum concentration can be described by two-compartment model following a single IM injection. The pharmacokinetic revealed that the values of distribution half-life ($T_{1/2alfa}$), maximum serum concentration (C_{max}), area under the curve ($AUC_{0-\infty}$) and mean resident time (MRT) were significantly lower in infected rabbits than in healthy rabbits. The volume of distribution (V/F) and Total body clearance of drug (CL/F) was significantly higher in infected than in healthy rabbits. Repeated IM administration of cefquinome revealed that liver retains the highest drug concentrations in both healthy and infected rabbits while the lowest drug concentrations were found in muscle. The recommended withdrawal time is 7 for healthy and 3 days for *K. pneumoniae* infected rabbits to be safe for human consumption. Finally; *K. pneumoniae* infection caused significant alterations in some pharmacokinetic parameters and tissue concentrations of cefquinome in rabbits.

INTRODUCTION

The economic importance of rabbits has increased dramatically for being raised for many objectives. Rabbits are considered a good source of palatable meat with high protein and low fat contents (**DalleZotte 2004**).

Klebsiella pneumoniae (*K. pneumoniae*) is a G-ve bacilli belonging to the family *of Enterobacteriaceae*. It is a widespread colonizer of the animal intestine, and it is able to survive and multiply in moist environment. This opportunistic bacterium can cause pneumonia and infections in the urinary tract, soft tissue, and skin in healthy animals (**Starlander and Melhus 2012**). *K. Pneumoniae* infection in rabbit is rare and usually associated with enteric form of infection (**Sumitha and Sukumar 2014**). In certain cases, the use of some antibiotics resulted in a significant decrease in the *K. pneumoniae*-numbers; nevertheless, treatment may be restricted if the bacterium produces an extended-spectrum β-lactamase (ESBL). ESBL-producing *K. pneumoniae*, which is reluctant to different antibacterial therapies (**Lautenbach et al. 2001; Zhang et al. 2014**), should be medicated by an extra-lactamase-stable anti-

Corresponding Author: Maha S. Abd Elhafeez, Department of chemistry, toxicology and feed deficiency, Animal Health Research Institute, Agricultural Research Center, Dokki, Giza, Egypt
E-mail address: mahasabry86_doctor@hotmail.com
DOI: 10.21608/EJAH.2021.133579

bacterial.

Cefquinome is a 4th generation cephalosporin antibiotic, which has been solely developed for veterinary use. It exhibitsbroad spectrum antimicrobial action and is stable against β-lactamases that are produced by a plurality of clinically important bacteria (**CVMP 1995 Bryskier, 1997**) so cefquinome seems to have a potential for treating infections caused by bacteria in rabbits. It had excellent activity against all the *Enterobacteriaceae* with MIC_{90} ranging from ≤ 0.12 to 2.0µg/mL (**Murphy et al. 1994**). It is mainly used for treatment of respiratory diseases in cattle, pigs, and horses, particularly in Europe (**CVMP 2003**).

The pharmacokinetics of cefquinome is characterized by rapid absorption and elimination, low protein binding and limited distribution in healthy rabbits (**Hwang et al. 2011; Shalaby et al. 2015**). However, no published data are available on the pharmacokinetics of cefquinome in diseased rabbits, particularly the ones infected with *K. Pneumoniae*. Understanding the pharmacokinetics of cefquinome in *K. Pneumoniae* infected rabbits may allow for design of future studies to explore the potential use of cefquinome in the treatment of *K. Pneumoniae* as there are few antibiotics that are effective in treating it in rabbits. The choice of antibiotics in rabbits is limited, as many oral antibiotics affect by the intestinal flora, resulting in *C. enterotoxaemia* (**Borriello and Carman 1983**). Therefore, alternative antibiotics, particularly the ones administered parentally, are needed to control this infection in rabbits. We aimed to investigate the influence of a moderate *K. Pneumoniae* infection on the pharmacokinetics and tissue residues of cefquinome in rabbits.

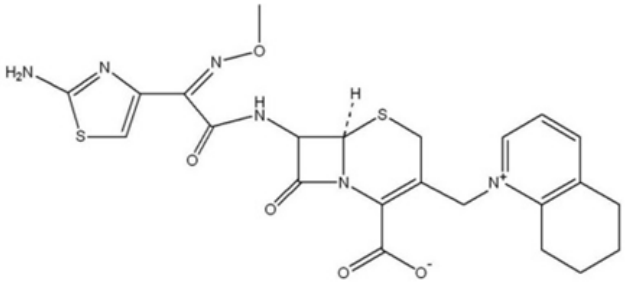

Figure (1): Chemical structure of cefquinome (**Uney et al. 2011**)

MATERIAL AND METHODS

Chemical reagents:

All reagents used during extraction and analysis were analytical grade. Acetonitrile, methanol, trichloroacetic acid (TCA), isooctane and de-ionized water (D.W) were HPLC grade (Fisher)

Tri-floro acetic acid (TFA) was spectrophotometric grade ≥ 99.9% (ALDRICH)

Ammonium acetate buffer 0.05M; pH5.

Cefquinome sulfate reference standard: VETRANAL, analytical standard, 80.9% was provided by Sigma Aldrich Chemical Co. (St. Louis, MO).

Cefquinome sulfate drug (COBACTAN®, 2.5%) was obtained from Intervet International Company, Cairo, Egypt. Cefquinome sulfate was administered I.M. at a dose of 2 mg / kg body weight (b.wt.) into the gluteal muscles (**Elazab et al. 2018**).

Instrument and chromatographic conditions:

The HPLC system (Agilent Technologies, 1200 series Japan) consists of a quaternary pump and a degasser to pump the mobile phase, an auto-sampler and a column oven. The detection was performed using a multi-wave detector (MWD) set at 267 nm. The column temperature was kept at 40°C. The reverse-phase chromatography was performed with an analytical Agilent C18 column (250

mm by 4.6 mm; internal diameter, 5 µm: Agilent Technologies). The optimized method used an isocratic mobile phase consists of water containing 0.1% TFA and ACN (90:10) (**Hwang et al. 2011**). Mobile phase was filtered, degassed by passage through a 0.45µm nylon filter (Millipore, Bedford, MA) under a vacuum, and was sonicated for 30 min. The flow rate was 1 ml/min, and the injection volume was 10 µl. The HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France) (**Elazab et al. 2016**).

Animals:

A total of 55 New Zealand white rabbits of both sexes was used in this study of mean (± SD) weight 1.5 ± 0.4 kg were used. Rabbits were housed in a specific pathogen-free room within the animal care facility, given free access to tap water and standard balanced rabbit chow. The animals were acclimated and did not receive any drug treatment for at least 15 days before the study. They were randomly divided into 5 groups, one group of 5 rabbits served as controls (they were used for preparation of blank sample and quality control samples for method validation); the remained 50 rabbits were grouped into 20 rabbits for pharmacokinetic study and 30 rabbits for residues depletion study.

Experimental Design: as in table (1)

Table (1) Grouping of experimental animals

Gp.	An. no.	Drug supplement	Dose	Administration route	Duration
A	5	Control group			
B	10	Cefquinome	2 mg/kg b.wt	IM	Once
C	10	Cefquinome	2 mg/kg b.wt	IM	Once daily for 3 days
D	15	*K. pneumoniae* + Cefquinome	0.25 ml (1×10^9 CFU/ ml) 2 mg/kg b.wt	endotracheal IM	……. Once
E	15	*K. pneumoniae* + Cefquinome	0.25 ml (1×10^9 CFU/ ml) 2 mg/kg b.wt	endotracheal IM	…….. Once daily for 3 days

CFU = colony forming unit

Artificial infection:

Enteropathogenic *Klebsiella Pneumoniae K1* (1×10^9 CFU/ ml) were kindly supplied by Serology Department, Animal Health Research Institute, Dokki, Giza, Egypt. Infection was infested by endotracheal inoculation of 0.25 ml of bacterial suspension (**Petraitiene et al. 2020**).

After endotracheal inoculation, rabbits suffered from unkempt appearance anorexia, sneezing, dyspnea, coughing, ocuolonasal discharge, diarrhea and fever (≥1.5°C) were observed before administration of cefquinome and during the experiment course; The mean body temperatures of infected group before infection and before drug administration were 38.6 ± 0.3 and 41.5 ± 0.4°C, respectively ($p<0.05$). Swabs from nasal discharges were cultured in beef infusion broth at 37°C for 24 h and plated on MacConkey agar. Confirmation of *K. pneumoniae* was based on morphology, gram staining and standard biochemical tests (**Chinedu et al. 2017**).

Susceptibility testing:

The Minimum inhibitory concentration (MIC) of cefquinome against *K. pneumoniae* was performed by a broth micro dilution method as described by CLSI approved standards (**CLSI, 2015**).

Sample collection:

For pharmacokinetic study (gp. B&C); about 0.5 ml blood were taken from the saphenous vein at different time intervals; 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72- and 96-hours

following injection. Samples were collected into heparinized tubes, and then centrifuged (1500xg for 10 min) and the serum was harvested and stored frozen at -20°C until analyzed by HPLC assay.

For residue depletion study (gp. D&E); three rabbits were slaughtered at 1st, 3rd, 5th, 7th, 9thand 14thday following the last dose. Samples from liver, muscle and kidney were taken from slaughtered rabbits for drug assay. One grams of tissue specimens was weighed and stored frozen at -20°C until assayed for concentrations of cefquinome (marker residues) using HPLC.

Stock solutions and standards preparation:

A standard stock solution of cefquinome was prepared by weighing of the standard with subsequent dissolution in water, obtaining a concentration of 1mg/ml; this solution was stored at -20°C. The stock solution was diluted quantitatively with water for the preparation of calibration standards and quality control (QC) samples.

Cefquinome calibration standards were prepared fresh daily in blank rabbit serum at concentrations of 0.05, 0.10, 0.20, 0.50, 1, 2, 5 and 10µg/ml for serum analysis. The cefquinome calibration curve was prepared at concentrations of 0.05, 0.1, 0.2, 0.5, 1 and 2 µg/gm in blank muscle, liver and kidney for residue analysis. In the same manner, QC samples with cefquinome in serum at low (50 ng/ml), medium (500 ng/ml), and high (5000 ng/ml), for muscle (25, 50, 100 ng/gm), for liver (50, 100, 200 ng/gm) and for kidney (100, 200, 400 ng/gm) concentrations were freshly prepared to evaluate the recovery and precision of this HPLC method. Prior to HPLC analysis, these calibration standards and QC samples were processed according to the method that is summarized as follows.

Serum sample extraction:

Aliquots of calibrators, QC samples, and other serum samples (200 µl) were added to 100 µl of 5% (TCA) was added and mixed for 10 s, then centrifugation at 4,000 x g for 10 min. After centrifugation, the clear supernatant transferred into HPLC vial (**Elazab et al. 2018**).

Tissue sample extraction:

Weight 1 gm of tissue sample in 15 ml polypropylene centrifugal tube. Add 4 ml of 0.05 M ammonium acetate buffer (TAC5) pH 5 and 1 ml iso-octane, mix for few seconds, then centrifuge at 2400 x g/ 10 min. The isooctane was discarded and the supernatant was transferred to previously activated solid phase cartridges with 1 ml MEOH, 1 ml D.W and 1 ml TAC5; then cartridges was washed using 1 ml TAC5, 1 ml D.W, then 1 ml 10% ACN. Air was pass via the cartridge for 5 min. elution by 1 ml elution solution 20% ACN; at a flow rate of 3 ml/min, the elute was evaporated at 50°C under nitrogen stream till complete dryness, then reconstituted in 500 µl D.W, filtration with 0.45 um Acrodiscs before injection into HPLC system (**Elazab et al. 2016**).

HPLC method validation (USP 2019):

This method was validated following AOAC International guidelines (**AOAC 2013**) for single-laboratory validation and United States Pharmacopeia Chapter 1225, Validation of Compendial Procedures and Chapter 621, Chromatography (**USP 2019**).

Selectivity and specificity:

It was evaluated by analyzing blank rabbit serum, muscle, liver, kidney and spiked rabbit serum, muscle, liver and kidney with cefquinome; chromatograms were visually examined for chromatographic interference from endogenous compounds.

Linearity and range:

These were evaluated by a calibration curve in the range of 0.02 to 10 µg/ml cefquinome in serum, and in the range of 0.05 to 2µg/gm in different matrixes. Triplicate injections of each level. Calibration curves were used to calculate the linear regression equation and the correlation coefficient.

The linear equation: y = ax +/- b, where *y* is the peak area, *x* is the concentration (ppm). *a is* the slope and *b* is intercept. The correlation coefficient (R^2) was calculated for each standard curve.

The limits of detection (LOD) and quantification (LOQ): were calculated using SD of intercept (S);[LOD = 3.3*S/a & LOQ = 10* S/a].

Precision and recovery: QC samples were analyzed for the determination of intra-day precision, inter-day precision and recovery %.
The intra- and inter-day precision of the assay were determined by coefficient of variation (CV) = (mean/SD) * 100.
The recovery percent = (Obtained concentration/ Theoretical concentration) *100.

System Suitability Test (SST): It is generally performed to evaluate the suitability and effectiveness of the entire chromatographic system not only prior to use but also during the time of analysis. The SST parameters which are investigated are retention time (RT), symmetry, theoretical number of column (N) and tailing factor (T).

Statistical Analysis:

Data were expressed as mean ± S.E. The obtained data were evaluated by two-tailed T-Test using the commercially available software package (SPSS Inc., version 22.0, Chicago, IL, USA) to express the differences between groups (**Morgan et al. 2019**) The differences were considered significant when $P<0.05$. The pharmacokinetic parameters were analyzed by PKsolver. An add-in program for Microsoft Excel, version 2, and other parameters were calculated according to (**Zhang et al. 2010**).

RESULTS

Susceptibility testing:

Minimum inhibitory concentration (MIC) of cefquinome was ≤ 0.125 µg/ml for *K. pneumoniae*.

Method validation and verification:

The analytical method linearity, range, LOD, LOQ, recovery and intraday & inter-day precision: the obtained results were summarized in table (2).

Table (2) Validation sheet of HPLC method

Parameter	Serum	Muscle	Kidney	Liver
Range (ppm)	0.05-10 µg/ml	0.05-2 µg/gm		
Retention time (min.)	14.242			
Regression equation	y = 88.176x + 0.2011	y = 77.794x + 0.6644	y = 77.794x + 0.6644	y = 67.672x + 1.4513
Correlation coefficient (R^2)	0.9999	0.9998	0.9998	0.9990
LOD (ppm)	0.0093	0.006	0.015	0.0133
LOQ (ppm)	0.027	0.018	0.045	0.040
Recovery %	98-101	90-97	92-105	94-102
Intra-day precision (CV %)	0.29	0.45	0.73	1.81
Inter-day precision (CV %)	0.94	1.3	1.73	2.63

LOD = Level of Detection LOQ = Level of Quantitation
CV = coefficient variance

Specificity*:* The equilibrated chromatograms of cefquinome either in serum, muscle, liver, and kidney samples were demonstrated specific at retention time 14.2 min showing no interference between the extracted different spiked matrixes and pure standard (**Figs, 2-6).**

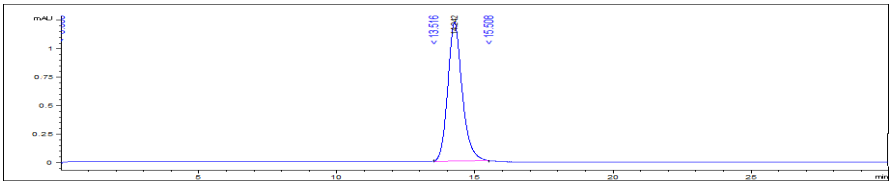

Fig. (2): Chromatogram showing pure standard of cefquinome at 0.5 ppm

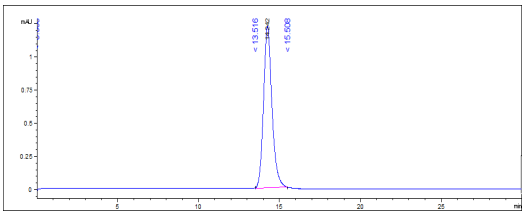

Fig. (3): Chromatogram showing cefquinome in serum at 0.5 ppm

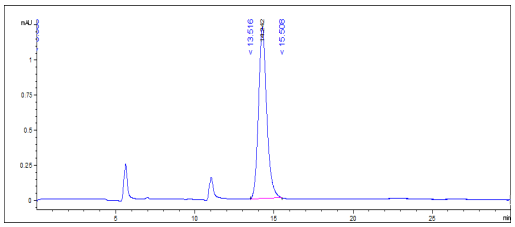

Fig. (4): Chromatogram showing cefquinome in muscle at 0.5 ppm

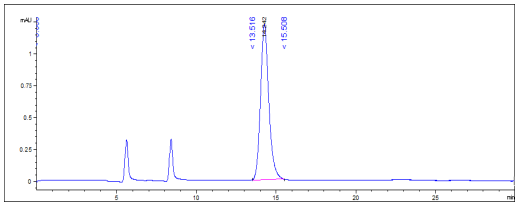

Fig. (5): Chromatogram showing cefquinome in liver at 0.5 ppm

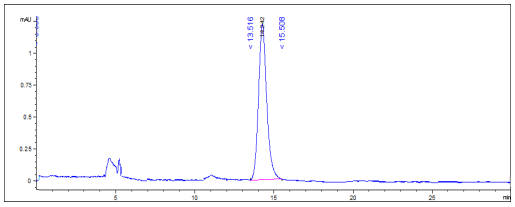

Fig. (6): Chromatogram showing cefquinome in kidney at 0.5 ppm

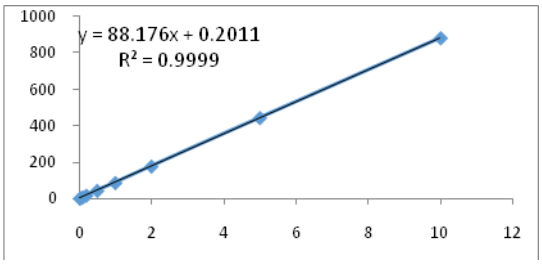

Fig. (7): Standard curve cefquinome in serum

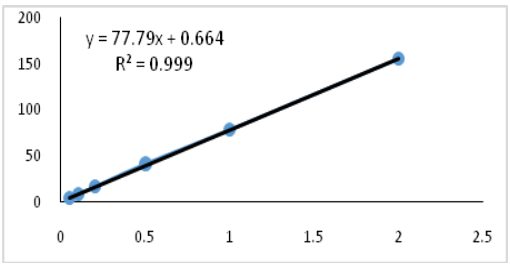

Fig. (8): Standard curve cefquinome in muscle

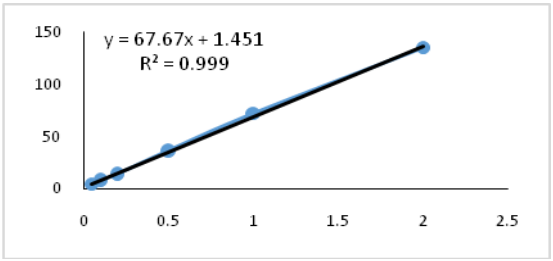

Fig. (9): Standard curve cefquinome in liver

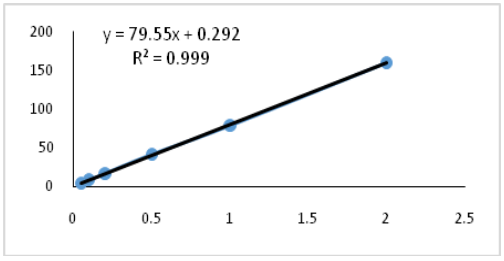

Fig. (10): Standard curve cefquinome in kidney

System suitability test (SST): The limits which are considered for the SST parameters and results of the present study are listed in table **(3).**

Table (3) SST parameters for cefquinome at a concentration of 0.1 ppm (n=6):

		Serum	Muscle	Liver	Kidneys	Acceptance criteria
Retention time (RT)	mean	14.242	14.24	14.23	14.2	
	RSD%	0.01	0.02	0.01	0.01	RSD <1.0%
Tailing factor (Tf)	mean	1.02	1.02	1.031	1.04	
	RSD%	0.07	0.04	0.02	0.026	≤2.0
Theoretical plates (N)	mean	6556	5435	6543	5423	>2000
	RSD%	1.9	1.9	1.36	1.6	
Symmetry factor	mean	0.45	0.56	0.67	0.63	≤1.0
	RSD%	0.01	0.001	0.01	0.01	≤1.0

Pharmacokinetics results:

After intramuscular injection of cefquinome, there were no observed adverse effects such as signs of pain, tissue irritation, or lameness.

The cefquinome concentrations in healthy and *K. pneumonia*-infected rabbit's serum following single IM administration are illustrated in table (4);the concentrations in serum of healthy rabbits were higher significantly at most time points than experimentally infected ones figure (7).

Table (4) Cefquinome levels in healthy- and *K. pneumonia*-infected rabbit's serum (µg/ml) after single IM injection of 2 mg/kg of cefquinome Sulfate

Time (hr)	Healthy rabbits	*K. pneumonia*-infected rabbits
0.25	4.68 ± 0.4	3.22 ± 0.34
0.5	5.69 ± 0.49	3.94 ± 0.41
1	5.38 ± 0.46	3.69 ± 0.38*
2	4.48 ± 0.39	3.10 ± 0.4
4	3.05 ± 0.28	1.98 ± 0.26*
6	1.73 ± 0.16	1.03 ± 0.14*
8	0.67 ± 0.06	0.2 ± 0.03*
10- 72	ND	ND

ND= not detectable

Data are expressed as mean ± SE (n = 10).

* Significant at $p < 0.05$ using t- test.

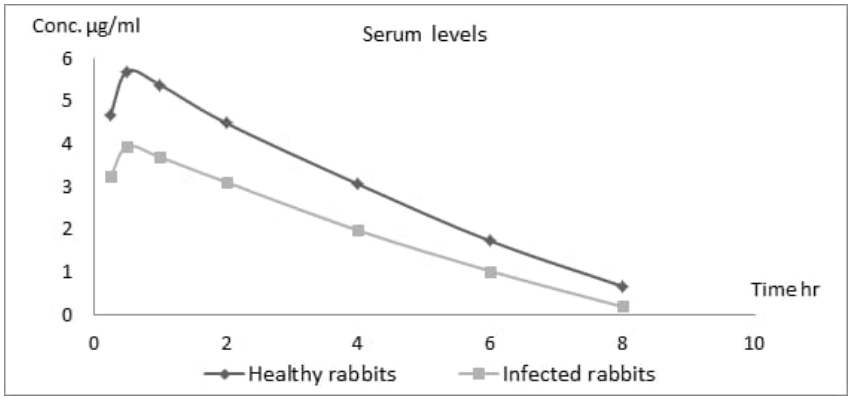

Fig. (7) Cefquinome levels in healthy-and K. pneumonia-infected rabbit's serum after single IM injection of 2 mg/kg of cefquinome Sulfate

The calculated pharmacokinetic parameters are shown in Tables (5); cefquinome was rapidly absorbed from injection sites in both healthy and *K. pneumonia* -infected rabbits with C_{max} (5.28 and 3.95 µg/ml, respectively) achieved at T_{max} 0.64 and 0.59 hr, respectively. Cefquinome was not detected in serum of healthy and *K. pneumonia* -infected rabbits at ≥ 8 hr after IM injection of cefquinome.

Table (5) The Pharmacokinetic parameters of cefquinome after single IM injection of (2 mg/kg) in healthy and *K. pneumonia* infected rabbits

Parameters	Unites	Healthy rabbits	*K. pneumonia*-infected rabbits
$T_{1/2Alpha}$	h	0.81 ± 0.003	0.49 ± 0.001*
$T_{1/2Beta}$	h	2.94 ± 0.02	2.8 ± 0.052
V/F	(ml/kg)	0.33 ± 0.01	0.44 ± 0.03*
CL/F	(ml/h/kg)	0.077 ± 0.001	0.11 ± 0.01*
T_{max}	h	0.64 ± 0.001	0.59 ±0.01*
C_{max}	µg/ml	5.28 ± 0.1	3.95 ± 0.39*
AUC_{0-t}	µg/ml*h	21.87 ± 0.45	15.7 ± 1.7*
AUC_{0-inf}	µg/ml*h	26 ± 0.58	18.37 ± 2.13*
AUMC	µg/ml*h^2	115.4 ± 3.1	77.7 ± 10.36*
MRT	h	4.44 ± 0.03	4.21 ± 0.07*

Data are expressed as mean ± SE (n = 10).
* Significant at $p < 0.05$ using t- test.

$T_{1/2Alpha}$: disposition half life; $T_{1/2Beta}$: Terminal half life; V/F: apparent volume of distribution during terminal phase; CL/F: apparent total clearance of drug from plasma after oral administration; T_{max}: time of reach maximum plasma concentration; C_{max}: maximum plasma concentration; AUC_{0-t}: area under the plasma concentration time curve from time zero to 8 hr; AUC_{0-inf}: area under the plasma concentration time curve from time zero to infinity; AUMC: area under the first moment of plasma concentration time curve.
MRT: mean residence time.

Results of tissue depletion study:

The cefquinome concentrations in healthy and *K. pneumonia*-infected rabbit's tissue following single IM administration are illustrated in table (6).

Table (6) The tissue residues of cefquinome (µg/gm) after single IM injection of (2 mg/kg) in healthy and *K. pneumonia*-infected rabbits

		1st	3rd	5th	7th	9th	15-21	MRL
Muscle	Healthy	0.02±0.002	Nd	Nd	Nd	Nd	Nd	0.05
	Infected	Nd	Nd	Nd	Nd	Nd	Nd	
Liver	Healthy	0.126±0.02	0.031±0.04	Nd	Nd	Nd	Nd	0.1
	Infected	0.05±0.003*	Nd	Nd	Nd	Nd	Nd	
Kidney	Healthy	0.76±0.02	0.39±0.02	0.31±0.01	0.19±0.01	0.06±0.004	Nd	0.2
	Infected	0.36±0.02*	0.19±0.01*	0.15±0.01*	0.09±0.01*	Nd	Nd	

MRL = Maximum Residue Level
Data are expressed as mean ± SE (n = 3).
* Significant at $p < 0.05$ using t- test. Nd < LOD.

DISCUSSION

Following IM administration of cefquinome in a single dose (2 mg/kg b.wt.) to healthy and experimentally infected rabbits, the drug serum concentration can be described by two-compartment model, this was previously reported by **Elazab et al. (2018**) in rabbits**,** while it was three-compartment in chickens (**El-Sayed et al. 2015**).

The values of distribution half-life ($T_{1/2alfa}$), maximum serum concentration (C_{max}), area under the curve ($AUC_{0-\infty}$) and mean resident time (MRT) were significantly lower in infected rabbits than in healthy rabbits. Volume of distribution (V/F) and Total body clearance of drug (CL/F) was significantly higher in infected than in healthy rabbits. These changes in pharmacokinetic parameters in *K. pneumonia*-infected rabbits could be due to endotoxin- induced vasodilatation and increased cardiac output which would lead to an increase in glomerular filtration rate. These cardiovascular changes could be due to the fever associated with *K. pneumonia* infection (**Ganzinger and Haslberger, 1985; McColm et al. 1986; Goudah et al. 2006**).

Similarly, **Elazab et al. (2018)** found that the $T_{1/2\,\lambda z}$, $AUC_{0-\infty}$, $AUMC_{0-\infty}$ and MRT were significantly lower in infected rabbits than in healthy rabbits. The CL/F was significantly higher in infected than in healthy rabbits.

Time to reach the maximum concentration T_{max} after IM administration in infected rabbits was 0.59 ± 0.01 h significantly lower than in healthy one 0.64 ± 0.001 h, and clearance CL/F was increased significantly in infected rabbits; Relatively lower T_{max} and higher CL/F demonstrate the rapid elimination of the drug in infected rabbits.

The present study showed that the $T_{1/2Beta}$of cefquinome following a single IM injection in healthy rabbits was 2.94 hr. In contrast, the $T_{1/2\,\lambda z}$ of cefquinome in the present study was higher than its corresponding value in rabbits administered with the same dose IM (1.04 hr) by **Hwang et al. (2011)** and was lower than its corresponding value in rabbits administered with the same dose IM (0.722 hr) by **Elazab et al. (2018)**. These differences could be related to the selected dose in this study, different rabbit's breeds, species and age variations which could affecting the degree of protein binding of the drug and/or due to the difference in the method used for assaying of the drug.

In the present study, Minimum inhibitory concentration (MIC) of cefquinome was ≤ 0.125 µg/ml for *K. pneumoniae* more than 0.030 µg/ml obtained by **Zhang et al. (2014**) and less than 0.25 µg/ml obtained by **Shan and Wang (2017)**. In the present study, at 8 hr post IM administration of 2 mg/kg cefquinome sulfate in *K. pneumoniae* -infected rabbits, the serum cefquinome was less than the determined MIC, therefore, 8 hr dosing interval is recommended for *K. pneumoniae* -infected rabbits to achieve optimal effectiveness against these bacteria. This dose regimen would be inconvenient and impractical in the treatment of *K. pneumoniae* infection in rabbits. Therefore, further study is recommended to use higher IM doses of cefquinome sulfate than 2 mg/kg (e.g., 4 or 6 mg/kg) in rabbits to reach a more convenient and practical dose interval in this species to treat *K. pneumoniae* infection.

In the present investigation, repeated IM administration of cefquinome revealed that liver retains the highest drug concentrations in both healthy and infected rabbits while the lowest drug concentrations were found in muscle.

These results are slightly agreed with that reported by **Gaber (2005), El-Sayed et al. (2015)** in chickens, **Elazab et al. (2018)** in rabbits. These findings suggested that cefquinome is excreted mainly via the kidney (**Limbert et al. 1991; San-Martin et al. 1998**).

The experimental infection caused significant reduction in tissue concentrations of cefquinome in rabbits. The MRLs permitted by the European Agency for The Evaluation Of Medicinal Products Committee For Veterinary Medicinal Products, for cefquinome are 50, 100 and 200 µg/Kg in muscle, liver and kidney; respectively of bovine and porcine (**CVMP 1995**). The recommended withdrawal time is 7 in healthy rabbits and 3 days in *K. pneumonia*-infected rabbits to be safe for human consumption.

CONCLUSION:

This study showed that *K. pneumonia* infection caused significant alterations in some pharmacokinetic parameters and tissue concentrations of cefquinome in rabbits. Based on

these findings, it is recommended that in *K. pneumonia* infected rabbits, cefquinome sulfate could be injected IM at 2 mg/kg every 8 hr to treat the infection. This dose regimen would be inconvenient and impractical for treating this infection in rabbits. After repeated IM administrations of cefquinome (2 mg/kg b.wt.) once daily for 3successive days, cefquinome was highly concentrated in the kidney followed by the liver, while dabs were detected in the muscle. Based on the MRLs established by regulatory agencies and statistical method suggested by EMEA, treated rabbits must not be slaughtered before 3 days from last dose of repeated administration of cefquinome to withdraw the drug residues from all tissues of treated rabbits.

Acknowledgment
We would like to thank Dr. Amany El- Bialy; Chief Researcher, Serology Unit, Animal Health Research Institute, Dokki, Giza on the supplying of *K. pneumonia* bacteria and confirmation of infection.

Conflict of Interest
The authors declare no conflict of interests.

References

AOAC International. 2013. Appendix K: Guidelines for dietary supplements and botanicals – Part I AOAC guidelines for single -laboratory validation of chemical methods for dietary supplements and botanicals. Official Method of Analysis of AOAC International, 1–15.

Borriello SP, Carman J. 1983. Association of iota- like toxin and *Clostridium spirojorme* with both spontaneous and antibiotic- associated diarrhea and colitis in rabbits. . Clin. Microbiol. (17): 414– 418.

Bryskier A. 1997. New concepts in the field of cephalosporins: C3′ quaternary ammonium cephems (Group IV). Clin. Microbiol. and Infect. (3): s1-s6.

Chinedu OC, Regina AN, Charles EC, Nnaemeka AI. 2017. Detection of Carbapenem-Resistant *Klebsiella Pneumoniae* Isolates from Clinical Specimens in Nnamdi Azikiwe University Teaching Hospital, Nnewi. Int. J. Cur. Res. Rev.| 9(10): 44.

CLSI WL. 2015. M02-A11: Performance standards for antimicrobial disk susceptibility tests; Approved Standard.

CVMP. 1995. Cefquinome. Summary report. EMEA/MRL/005/95.

CVMP. 2003. Cefquinome (Extension to Horses). Summary Report (3). EMEA/ MRL/883/03-FINAL. European Agency for the Evaluation of Medicinal Products, London, UK.

DalleZotte A. 2004. Dietary advantages: rabbit must tame consumers. Viandesetproduits Carnes 23(6): 161– 167.

Elazab ST, Gabr MG, Amer MS, El-Nabtity SM, Hsu WH. 2016. Depletion of cefquinome from rabbit tissues. International Journal of Innovation and Applied Studies 18 (3): 743.

Elazab ST, Schrunk DE, Griffith RW, Ensley SM, Dell'Anna G, Mullin K, Hsu WH. 2018. Pharmacokinetics of cefquinome in healthy and *Pasteurella multocida*-infected rabbits. J. Vet. Pharmacol. Therap. 41(3): 374-377.

El-Sayed MG, El-Komy AA, Mobarez EA, El-Mahdy AM. 2015. Pharmacokinetics and Tissue residues of cefquinome in normal and *Salmonella entretidis* infected chickens. World J. Pharmacy Pharmaceut. Sci. 10(4): 1974– 1987.

Gaber M. 2005. Pharmacokinetics of cefquinome and tissue concentrations in broilers. Bull. Fac. Pharm., Cairo Univ. 43 (2).

Ganzinger U. and Haslberger A. 1985. Pharmacokinetics of cephalosporins in normal and septicemic rabbits. Antimicrob. Agents Chemoth. (28): 473– 477.

Goudah A, Mouneir SM, Shim JH, Am AEA. 2006. Influence of endotoxin induced fever on the pharmacokinetics of intramuscularly administered cefepime in rabbits. J. Vet. Sci. 7(2): 151– 155.

Hwang YH, Song IB, Lee HK, Kim TW, Kim MS, Lim JH, Yun HI. 2011. Pharmacokinetics and bioavailability of cefquinome in rabbits following intravenous and intramuscular administration. J. Vet. Pharmacol. Therap. (34): 618– 620.

Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. 2001. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on out-

comes. Clinl Infect. Dis. 32(8): 1162-1171.
Limbert M, Isert DIETER, Klesel NORBERT, Markus ASTRID, Seeger, KARL, Seibert, GERHARD, Schrinner, ELMAR. 1991. Antibacterial activities in vitro and in vivo and pharmacokinetics of cefquinome (HR 111V), a new broad-spectrum cephalosporin. Antimicrob. Agents Chemoth. 35(1): 14-19.
Mc-Colm AA, Shelley E, Ryan DM, Acred P. 1986. Evaluation of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia: comparison with other antibiotics and measurement of its penetration into respiratory tissues and secretions. J Antimicrob. Chemother. (18): 599–608.
Morgan GA, Barrett KC, Leech NL, Gloeckner GW. 2019. IBM SPSS for introductory statistics: Use and interpretation. Routledge.
Murphy SP, Erwin ME, Jones RN. 1994. Cefquinome (HR 111V) in vitro evaluation of a broad-spectrum cephalosporin indicated for infections in animals. Diag. Microbiol. Infect. Dis. 20(1): 49-55.
Petraitiene R, Petraitis V, Kavaliauskas P, Maung BBW, Khan F, Naing E, Stakauskas R. 2020. Pharmacokinetics and Efficacy of Ceftazidime-Avibactam in the Treatment of Experimental Pneumonia Caused by *Klebsiella pneumoniae* Carbapenemase-Producing *K. pneumoniae* in Persistently Neutropenic Rabbits. AntimicrobAgents Chemoth. 64(4).
San Martin BN, Bataglia J, Hernandez P, Quiroz A, Canon H. 1998. Absorption and excretion of cefquinome in coho salmon (Oncorhynchuskisutch) in freshwater at 10 degrees C. Zentralblatt fur Veterinarmedizin. Reihe A. 45(10), 615.
Shalaby MA, Goudah A, Gihan MK, Hassan AS. 2015. Disposition kinetics of cefquinome in healthy rabbits following intramuscular and oral administration. World J. Pharmacy and Pharmaceu. Sci. (4): 263–274.
Shan Q, Wang J. 2017. Activity of cefquinome against extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in neutropenic mouse thigh model. J. Vet. Pharmacol. Therap. 40(4): 392-397.
Starlander G, Melhus Å. 2012. Minor outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in an intensive care unit due to a contaminated sink. J. Hosp. Inf. 82(2): 122-124.
Sumitha P, Sukumar K. 2014. An outbreak of *Klebsiella pneumoniae* infection in a rabbit farm. Inter. J. Current Microbi. Appl. Sci. 3 (11): 789– 790.
USP C. 2019. 1225, Validation of compendial procedures & 621, Chromatography. Rockville, Rockville, MD: United States Pharmacopeia.
Zhang B, Gu X, Li X, Gu M, Zhang N, Shen X, Ding H. 2014. Pharmacokinetics and ex-vivo pharmacodynamics of cefquinome against *Klebsiella pneumonia* in healthy dogs. J. Vet. Pharmacol. Therap. 37(4): 367-373.
Zhang Y, Huo M, Zhou J, Xie S. 2010. PK-Solver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel.