PK-PD Modeling and Optimal Dosing Regimen of Acetylkitasamycin against *Streptococcus suis* in Piglets

Anxiong Huang 1,2,†, Feng Mao 1,2,†, Lingli Huang 1,2, Shuyu Xie 1,2, Yuanhu Pan 1,2, Wei Qu 1,2, Guyue Cheng 1,2, Zhenli Liu 1,2, Zonghui Yuan 1,2, Dapeng Peng 1,2,* and Haihong Hao 1,2,†

1 National Reference Laboratory of Veterinary Drug Residues (HZAU) and MOA Key Laboratory for Detection of Veterinary Drug Residues, Wuhan 430070, China; anxionghuang@webmail.hzau.edu.cn (A.H.); maof890518@126.com (F.M.); huanglingli@mail.hzau.edu.cn (L.H.); sxxy1@126.com (S.X.); panyuanchu@mail.hzau.edu.cn (Y.P.); qw@mail.hzau.edu.cn (W.Q.); chenggyuye@mail.hzau.edu.cn (G.C.); liuzhili009@mail.hzau.edu.cn (Z.L.); yuan5802@mail.hzau.edu.cn (Z.Y.)

2 MOA Laboratory for Risk Assessment of Quality and Safety of Livestock and Poultry Products, Wuhan 430070, China

* Correspondence: pengdapeng@mail.hzau.edu.cn (D.P.); haihong_hao@aliyun.com (H.H.);
Tel.: +86-27-158-7181-2208 (H.H.); Fax: 0086-27-87672232 (H.H.)
† These authors contributed equally to this work.

**Abstract:** *Streptococcus suis* (*S. suis*) causes severe respiratory diseases in pigs and is also an important pathogen causing hidden dangers to public health and safety. Acetylkitasamycin is a new macrolide agent that has shown good activity to Gram-positive cocci such as *Streptococcus*. The purpose of this study was to perform pharmacokinetic–pharmacodynamic (PK-PD) modeling to formulate a dosing regimen of acetylkitasamycin for treatment of *S. suis* and to decrease the emergence of acetylkitasamycin-resistant *S. suis*. The minimal inhibitory concentration (MIC) of 110 *S. suis* isolates was determined by broth micro dilution method. The MIC~50~ of the 55 sensitive *S. suis* isolates was 1.21 μg/mL. The strain HB1607 with MIC close to MIC~50~ and high pathogenicity was used for the PK-PD experiments. The MIC and MBC of HB1607 in both MH broth and pulmonary epithelial lining fluid (PELF) was 1 and 2 μg/mL, respectively. The liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was used to determine the concentration change of acetylkitasamycin in piglet plasma and PELF after intragastric administration of a single dose of 50 mg/kg b.w. acetylkitasamycin. The PK parameters were calculated by WinNolin software. The PK data showed that the maximum concentration (Cmax), peak time (Tmax), and area under the concentration–time curve (AUC) were 9.84 ± 0.39 μg/mL, 4.27 ± 0.19 h and 248.58 ± 21.17 h·μg/mL, respectively. Integration of the in vivo PK data and ex vivo PDL data, an inhibition sigmoid Emax equation was established. The dosing regimen of acetylkitasamycin for the treatment *S. suis* infection established as 33.12 mg/kg b.w. every 12 h for 3 days. This study provided a reasonable dosing regimen for a new drug used in clinical treatment, which can effectively be used to treat *S. suis* infection and slow down the generation of drug resistance.

**Keywords:** *Streptococcus suis*; acetylkitasamycin; PK-PD; dosing regimen; PELF

1. **Introduction**

*Streptococcus suis* (*S. suis*) is an important pathogen in swine, and can cause serious respiratory disease, resulting in great economic losses to the swine industry worldwide each year [1]. As a zoonotic pathogen *S. suis* can even cause human death [2]. Every year, a large number of antibiotics, including macrolides, are used for the treatment of respiratory disease. However, drug resistance has emerged as a result of the unreasonable use of drugs [3].

Macrolides are widely used in veterinary medicine to prevent and treat respiratory diseases and necrotic enteritis [4]. Acetylkitasamycin is a product of kitasamycin acetylation [5]. It has a similar spectrum of antibiotic activity to kitasamycin, but has superior...
pharmacokinetic (PK) properties and better palatability. Acetylkitamycin mainly has good antibacterial effect on Gram-positive cocci (e.g., Streptococcus and Staphylococcus aureus) and mycoplasma [6,7]. Many macrolides can accumulate in the lungs in order to achieve higher drug concentrations [8,9], so if acetylkitamycin can achieve higher concentrations than plasma, it would be suitable for the treatment of lung infections. The pharmacokinetics of acetylkitamycin in porcine ileum content has been revealed [10]; however, its PK in the respiratory tract remains largely unknown.

To reduce the occurrence of drug resistance, a reasonable dosing regimen is necessary. Pharmacokinetic–pharmacodynamic (PK-PD) properties are very important in the determination of dosing regimens of drugs [11,12]. Three parameters, including T > MIC, AUC/MIC and C\textsubscript{max}/MIC, are commonly used in PK-PD modeling [13]. T > MIC is used for time-dependent drugs, such as β-lactam antibiotics [14]; AUC/MIC is used for time-dependent drugs with significant PAE, such as glycopeptides and macrolides [15,16]; and AUC/MIC or C\textsubscript{max}/MIC are used for concentration-dependent drugs, such as aminoglycosides and fluoroquinolones [17,18].

Bronchoalveolar lavage is widely used for collecting samples from the respiratory tract [19], and it is also commonly used to collect pulmonary epithelial lining fluid (PELF) to study the PK of drugs in pigs [20]. The bronchoalveolar lavage has a large advantage in taking pulmonary samples, as compared to homogenized lung tissue [21], cotton swab [22], microdialysis [23], imaging techniques, and other methods [24].

When a new drug is used in clinical treatment, a reasonable dosing regimen is necessary. A reasonable dosing regimen can not only achieve the maximum therapeutic effect in clinical treatment, it can also slow down the occurrence of drug resistance and prolong the effective time of the drug. In this study, on the basis of a combined PK-PD study of acetylkitasamycin against S. suis, a reasonable dosing regimen of acetylkitasamycin for the treatment respiratory infection caused by S. suis was formulated to provide medication guidance for controlling clinical S. suis infection.

2. Materials and Methods

2.1. Drug and Reagents

Acetylkitasamycin was provided by Hai Na Chuan (a pharmaceutical company in Guangdong, China), and has five main components: A\textsubscript{6}A\textsubscript{7}, A\textsubscript{5′}, A\textsubscript{4}A\textsubscript{5}, A\textsubscript{1}A\textsubscript{3}, A\textsubscript{13}. A single product of acetylkitasamycin (A\textsubscript{6}A\textsubscript{7}, A\textsubscript{5′}, A\textsubscript{4}A\textsubscript{5}, A\textsubscript{1}A\textsubscript{3}, A\textsubscript{13}) was isolated and prepared by the National Veterinary Drug Residue Reference Laboratory of Huazhong Agricultural University, and the purity of all products was ≥90%. Acetonitrile, formic acid, and methanol were purchased from TEDIA (Fairfield, OH, USA). Normal hexane and ethyl acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). In this experiment, all chemicals used were of analytical grade or higher. All water used was de-ionized water (Milli-Q Millipore Corp, Bedford, MA, USA).

2.2. Animals

Six weaned binary hybrid castrated healthy piglets weighing 20 ± 2 kg were purchased from Huazhong Agricultural University pig breeding farm. All the piglets were kept in the optimal environment. The piglets were fasted for 12 h before the experiments. All the animal experiments were approved by the Animal Ethics Committee of Huazhong Agricultural University (HZAUSW 2015-016) and the Animal Care Center, Hubei Science and Technology Agency in China (SYXK 2013-0044). All efforts were made to reduce the pain and adverse effects of the animals.

2.3. PD Study of Acetylkitasamycin against S. suis

2.3.1. Isolation and Identification of S. suis

From the year 2013 to 2015, 110 S. suis were isolated from pig farms in Hubei, Henan, Guangdong, Hebei, Jiangsu and Shandong provinces in China. The respiratory tract samples of pigs with respiratory diseases were collected, then inoculated into Tryptic Soy
Agar (TSA) medium supplemented with 5% fetal bovine serum. After culturing for 18–24 h under appropriate conditions, suspicious colonies were picked out for PCR identification. The primers required for PCR identification are designed based on the nucleotide sequence GDH of the specific gene of *S. suis* [25].

2.3.2. Determination of MIC, MBC, MPC and PAE

The minimal inhibitory concentration (MIC) of acetylkitasamycin and its five main components against *S. suis* strains were determined in MH broth and PELF by broth microdilution method according to the CLSI 2007 [26,27]. The *S. suis* ATCC 49619 and *E. coli* ATCC 25922 strains were used as the quality control strain for antibiotic susceptibility determination. According to the MIC\(_{50}\) values of sensitive strains, an *S. suis* HB1607 strain with its MIC similar with MIC\(_{50}\), was used for PD study of acetylkitasamycin.

The supernatant was sucked up from the MIC determination wells to the TSA in order to determine the minimal bactericidal concentration (MBC) [28]. The agar dilution method was used to determine the mutant prevention concentration (MPC) of acetylkitasamycin [28]. The 10\(^{10}\) CFU/mL bacterial were inoculated on the agar plates containing continuous concentrations of acetylkitasamycin (MIC, 2MIC, 4MIC, 8MIC, 16MIC, 32MIC) and cultured at 37 °C for 72 h, and the lowest concentration without bacterial growth was MPC.

Post-antibiotic effect (PAE) was estimated by incubating bacteria with drug for a period of time and then removal of drug [29]. First, the bacteria were incubated with 1MIC, 2MIC, 4MIC of drugs for 1 and 2 h; second, the drugs were removed by washing with new medium; third, 100 µL new incubation was sucked up at different time points and counted by plating on TSA. Then the recovery growth kinetic curves were established for computing the PAE. The PAE was calculated as follows: PAE = \(T - C\), where T is the time required for viable counts of bacteria to increase by 1 \(-\log_{10}\) CFU in drug removal phase, respectively; C is the time for untreated control.

2.3.3. In Vitro and Ex Vivo Bacterial Killing Curves

Prepare TSB (Tryptic Soy Broth with 5% fetal bovine serum) containing different concentrations of acetylkitasamycin (1/2MIC, 1MIC, 2MIC, 4MIC, 8MIC, 16MIC, 32MIC) for in vitro bacteria killing curves [30]. The different concentrations of drugs and bacteria (10\(^6\) CFU/mL) were incubated at 37 °C, 5% CO\(_2\). At each of the time points (0, 1, 2, 4, 6, 8, 12, 24 h), 100 µL of medium was sucked up, diluted with saline and coating on TSA, and the colony forming unit (CFU) changes after incubation in a 37 °C, 5% CO\(_2\) environment for 24 h were counted.

The ex vivo time-killing curves were estimated in PELF samples taken from piglets at different time points (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h) after intragastric administration with 50 mg/kg b.w. acetylkitasamycin [30]. The method was the same as that in an earlier in vitro study. Each concentration test was performed in triplicate.

2.4. PK Study of Acetylkitasamycin in Piglets

2.4.1. Animal Experiment and Sample Collection for PK Study

Acetylkitasamycin was administrated in six piglets with a single dose of 50 mg/kg b.w. by intragastric administration. After administration, 5 mL blood samples were collected through the anterior vena cava at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h. Plasma was separated from blood by centrifugation at 3500 rpm for 10 min.

To collect PELF samples, atropine (0.05 mg/kg) and propofol (9–15 mg/kg) were given intramuscularly and intravenously 30 min for anesthesia. Standardized Bronchoaveolar Lavage was performed as previously described [31,32], with an electronic fiber optic bronchoscope (Kangmei GU-180 VET, Zhuhai, China) inserted in the right middle lung lobe. A 50 mL volume of normal saline was instilled in the lobe, and was aspirated into a 50 mL centrifugal tube. The PELF samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h, and centrifuged at 800 rpm for 10 min.
2.4.2. Assay of Acetylkitasamycin and Kitasamycin Every Component in Plasma and PELF

The urea dilution method was used to determine the volume of PELF, as described previously [33]. To determine the urea concentration in lavage fluid samples, a biochemical analyzer machine (SYNCHRON CX4 PRO) was used. Estimation of the volume of PELF was done by the urea dilution method. The final concentration of acetylkitasamycin in PELF (C_{PELF}) was derived from the following equation: \[ C_{PELF} = \frac{C_{BAL} \times (\text{Urea}_{PLASMA} / \text{Urea}_{PELF})}{\text{Urea}}. \]

Quantitation of acetylkitasamycin and kitasamycin in every component of piglet plasma and PELF was conducted using the sensitive and selective high-performance liquid chromatographic mass spectrometry (LC-MS/MS) method [34]. The plasma specimens (0.25 mL) and BAL specimens (0.25 mL) were thawed and added to a 10 mL centrifuge tube with 0.75 mL acetonitrile, and vortexed for 3 min (12,000 r/10 min); 1 mL ethylacetate was added to the supernatant and vortexed for 3 min (12,000 r/10 min), and then the supernatant was taken out. The above operation process was repeated. The two supernatants were merged and blown dry with nitrogen. Then the residue was reconstituted in 0.25 mL solution (0.1% Formic acid water (60): Acetonitrile (40)).

All PK parameters of plasma and PELF were performed using WinNonlin software (version 5.2.1, Pharsight Corporation, Mountain View, CA, USA). Each piglet’s drug concentrations were depicted on semilogarithmic graphs to choose appropriate PK compartmental models.

Taking into account that acetylkitasamycin has several components, the weight coefficient was introduced in this study. Weight coefficient (w_i) can be customized for each component AUC_{0-\infty,i} in consideration of the total AUC_{0-\infty} ratio. Every monomer composition of acetylkitasamycin in the concentration of PELF was given their own weight coefficient, and then the total concentration was calculated (C_T).

\[
w_i = \frac{\text{AUC}_{0-\infty,i}}{\text{AUC}_{0-\infty}} (i = A_6A_7, A_5, A_4A_5, A_1A_3, A_{13})
\]

\[
\text{AUC}_{0-\infty} = \text{AUC}_{0-\infty A_6A_7} + \text{AUC}_{0-\infty A_5} + \text{AUC}_{0-\infty A_4A_5} + \text{AUC}_{0-\infty A_1A_3} + \text{AUC}_{0-\infty A_{13}}
\]

\[
C_T = w_{A_6A_7} \times C_{A_6A_7} + w_{A_5} \times C_{A_5} + w_{A_4A_5} \times C_{A_4A_5} + w_{A_1A_3} \times C_{A_1A_3} + w_{A_{13}} \times C_{A_{13}}
\]

2.5. PK-PD Integration and Modeling

All the parameters were calculated using WinNonlin 5.2 software. The surrogate parameters (C_{max}/MIC, AUC_{24h}/MIC, T > MIC) of PELF were determined after intragastric administration of acetylkitasamycin, used for in established vitro MIC and in vivo PK relationships.

The inhibitory sigmoid E_{max} model was used to establish the relationship between ex vivo AUC_{24h}/MIC ratio and the bacteria decrease in the PELF of piglets [16]. The model formula can be described as follows: \[ E = E_0 - \frac{\text{PD}_{\text{max}} C}{	ext{C}^N + \text{EC}_{50}^N}. \] E is the summary PD endpoint, and \( E_0 \) is the effect representing the value of the PD endpoint without drug treatment (i.e., the value of the summary endpoint when the PK-PD index is 0). C is one of the three PK-PD indices, as defined above, and PD_{max} is the maximum effect (in relation to \( E_0 \)) indicated by the plateau where further exposure does not result in further killing. EC_{50} is the magnitude of C that is needed to achieve 50% of E_{max} – E_0, and N is the sigmoidicity factor. The PD target under different efficiencies (E = 0, −3 and −4 (bacteriostasis, bactericidal and eradication)) was determined using the Sigmoid E_{max} equation [35].

2.6. Dosage Designation

Daily dose was calculated using the dosage equation: \[ \text{Dose} = \frac{\text{CL} \times (\text{AUC}_{24h}/\text{MIC}) \times \text{MIC}}{F \times f_u}, \] where CL is the clearance, AUC_{24h}/MIC is the targeted endpoint for optimal efficacy, f_u is free fraction of drug in plasma, F is the bioavailability factor(from 0 to 1) [36]. The f_u in epithelial lining fluid can be ignored, because of the low albumin levels in epithelial lining fluid [33].
To investigate the effect of different dosing regimens, the PD model indicates that the bacterial growth rate in the function of acetylkitasamycin concentration is combined with PK model, and simulations were performed with MlxPlore software (version 1.1.1, Lixoft, Orsay, France).

3. Results

3.1. PD Study of Acetylkitasamycin on S. suis

3.1.1. MIC of Acetylkitasamycin against S. suis Isolates

All the MIC of acetylkitasamycin against 110 S. suis were in the range of 0.25–128 μg/mL. MIC distribution of the acetylkitasamycin against 110 S. suis is shown in Figure 1. Non-linear least squares regression was used to fit a series distribution of log\(_2\)-transformed MIC data to a range of symmetrical 'bell-shaped' theoretical population distributions which was conducted in GraphPad Prism 5 software (Tables 1 and 2). The results manifested the smallest difference between the estimated and true number of isolates in the subset of 16 μg/mL (Table 2), so 16 μg/mL was set as the wild-type cutoff value. To select a sensitive strain, the wild-type cutoff value was set as interpretive criteria. The MIC\(_{50}\) and MIC\(_{90}\) of 110 strains were 9.10 μg/mL and 100.31 μg/mL, respectively. According to the interpretation criteria, which were set as described above, a total of 55 strains were sensitive strains, and the MIC\(_{50}\) and MIC\(_{90}\) were 1.21 μg/mL and 6.94 μg/mL, respectively (Table 3).

![Figure 1. Acetylkitasamycin MIC distribution of 110 S. suis strains isolated.](image)

### Table 1. Distribution Log\(_2\)MICs and cumulative distribution Log\(_2\)MICs.

| Parameter     | Distribution Log\(_2\)MICs |
|---------------|--------------------------|
|               | Counts | Cumulative |
|               | 0      | 1          | 2          | 3          | 4          | 5          | 6          | 7          |
|               | 0.25   | 0.5       | 1         | 2          | 4          | 8          | 16         | 32         | 64         | 128       |

| Parameter     | Distribution Log\(_2\)MICs |
|---------------|--------------------------|
|               | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|               | 7 | 4 | 18 | 6 | 6 | 7 | 6 | 1 | 38 | 17 |

### Table 2. Optimum non-linear least squares regression fitting of pooled MICs (μg/mL).

| Subset Fitted | Number of Isolates | Mean MIC (Log\(_2\)) | Standard Deviation (Log\(_2\)) |
|---------------|--------------------|----------------------|-------------------------------|
| True | Est | Diff | ASE | Est/ASE | 95% CI | Est | ASE | Est/ASE | 95% CI | Est | ASE | Est/ASE | 95% CI |
| ≤2  | 35  | 39  | 4 | 11.88 | 3.2 | -11.8, 19.90 | 0.569 | 0.634 | -0.9 | -8.835, 17.495 | 1.271 | 0.688 | 2.6 | 0.9, 9.659 |
| ≤4  | 41  | 42  | 1 | 4.678 | 8.9 | 21.77, 62.03 | -0.443 | 0.300 | -1.5 | -1.736, 0.8502 | 1.297 | 0.371 | 3.4 | 0.0, 2.896 |
| ≤8  | 48  | 47  | -1 | 4.492 | 10.4 | 33.65, 62.24 | -0.113 | 0.314 | -0.4 | -1.104, 0.8778 | 1.656 | 0.389 | 4.2 | -1.104, 0.87 |
| ≤16 | 54  | 54  | 0 | 4.549 | 11.8 | 41.35, 66.61 | 0.252 | 0.332 | 0.2 | -0.671, 1.176 | 2.037 | 0.417 | 4.8 | 0.878, 3.195 |
| ≤32 | 55  | 56  | 1 | 2.964 | 18.8 | 47.54, 62.78 | 0.328 | 0.245 | 1.3 | -0.305, 0.960 | 2.118 | 0.245 | 8.6 | -0.303, 0.96 |

Note: Est, non-linear regression estimate of value; Diff, estimate of N minus true; ASE, asymptotic standard error; Est/ASE, estimate divided by asymptotic standard error; CI, Confidence interval. *a*, this subset gave the smallest difference between the estimated and true number of isolates in the subset, and was therefore selected for estimates of the mean and standard deviation for the antibiotic–bacterium concentration.
Table 3. Susceptibilities of isolated S. suis strains.

| Antibiotic          | S. suis (110 strains) | S. suis (55 strains) |
|---------------------|-----------------------|----------------------|
| Acetylkitasamycin   | MIC<sub>50</sub> 9.10 | MIC<sub>50</sub> 100.23 | Range 0.25–128 | MIC<sub>50</sub> 1.21 | MIC<sub>90</sub> 6.94 | Range 0.25–16 |

Note: b, the total bacterial number; c, the susceptible strain based on this paper set interpretive criteria.

3.1.2. MIC, MBC, MPC and PAE of Acetylkitasamycin against S. suis HB1607

Through the toxicity experiment in mice the strain number HB1607 with serotype 2 which MIC close to MIC<sub>50</sub> was used to study the antimicrobial activity of acetylkitasamycin in vitro and ex vivo.

MICs of acetylkitasamycin in MHB and PELF against S. suis HB1607 were 1 µg/mL, MBC were 2 µg/mL and 4 µg/mL, respectively. The MICs of single components of acetylkitasamycin against S. suis HB1607 in MHB were both 1 µg/mL, and MBCs were both 2 µg/mL. In addition, the MICs of metabolites kitasamycin against S. suis HB1607 were both 2 µg/mL, MBCs were both 8 µg/mL. The MPC of acetylkitasamycin against S. suis HB1607 in MHB was 5 µg/mL. The PAE of acetylkitasamycin against S. suis HB1607 is shown in Table 4.

Table 4. The PAE of acetylkitasamycin against S. suis.

| Concentration (µg/mL) | Post-Antibiotic Effect (PAE) |
|-----------------------|-------------------------------|
|                       | Expose 1 h | Expose 2 h |
| 1MIC                  | 0.92        | 1.72        |
| 2MIC                  | 1.59        | 2.33        |
| 4MIC                  | 1.88        | 2.96        |

3.1.3. In Vitro and Ex Vivo Antimicrobial Activity

According to the MIC values, a series of concentrations of acetylkitasamycin was prepared to describe the killing curve. The curves were characteristically time dependent with significant PAE (Figure 2). Along with the extended time, the bacteria number decreased slowly, but the bactericidal activity was enhanced. In addition, when exposed to the higher concentrations (≥1 µg/mL) of acetylkitasamycin for 4 h, the bacteria decreased, but not to an undetectable level (<30 CFU).

![Figure 2. In vitro antibacterial of acetylkitasamycin against S. suis in MHB.](image)

The ex vivo killing curve results showed that acetylkitasamycin was also time-dependent (Figure 3), and was consistent with the in vitro killing curve. Along with time prolongation and the increase in concentration, the number of bacteria decreased sharply.
of A′ and A4A5 were detected in plasma with continuous concentrations. CmaxPELF/Cmaxplasma of A5′ was 31.54, AUCPELF/AUCplasma was 141.04; CmaxPELF/Cmaxplasma of A4A5 was 44.38, AUCPELF/AUCplasma was 241.65. In addition, the AUC, Cmax, Tmax in PELF were 248.58 h·μg/mL, 9.85 μg/mL and 4.27 h, respectively.

The concentrations of every component of acetylkitasamycin and its main metabolite kitasamycin in PELF were determined by LC-MS/MS after intragastric administration is shown in Figure 4.

Figure 4. Acetylkitasamycin concentrations in PELF-versus-time curves plotted semilogarithmically for data obtained after intragastric administration.
Table 5. PK of acetylkitasamycin and every component in plasma and PELF after intragastric administration (n = 6).

| Parameter | Acetylkitasamycin (Plasma) | Acetylkitasamycin (PELF) |
|-----------|-----------------------------|--------------------------|
| A | A₅ | A₄A₅ | A₄A₇ | A | A₄A₅ | A₁A₃ | A₃ | Total |
| α | 0.56 | ±0.04 | 0.46 | ±0.02 | 0.25 | ±0.01 | 0.3 | ±0.01 | 0.28 | ±0.02 | 0.25 | ±0.02 | 0.3 | ±0.02 | 0.27 | ±0.03 |
| β | 0.09 | ±0.01 | 0.19 | ±0.02 | 0.018 | ±0.04 | 0.022 | ±0.04 | 0.012 | ±0.01 | 0.017 | ±0.02 | 0.018 | ±0.02 | 0.016 | ±0.03 |
| T₁/₂₀₁ | 1.2 | ±0.08 | 1.49 | ±0.13 | 2.72 | ±0.09 | 2.29 | ±0.12 | 2.42 | ±0.28 | 2.75 | ±0.22 | 2.26 | ±0.16 | 2.54 | ±0.09 |
| T₁/₂₁₀ | 1.4 | ±0.21 | 1.5 | ±0.16 | 8.19 | ±0.63 | 6.57 | ±0.47 | 9.4 | ±2.9 | 34.93 | ±56.77 | 6.66 | ±1.49 | 7.25 | ±0.78 |
| T₁/₂α | 1.25 | ±0.13 | 1.5 | ±0.16 | 5.2 | ±0.11 | 2.31 | ±0.13 | 2.42 | ±0.25 | 2.75 | ±0.23 | 2.28 | ±0.17 | 2.62 | ±0.27 |
| T₁/₂β | 7.47 | ±0.54 | 3.56 | ±0.25 | 39.52 | ±3.27 | 32.16 | ±3.17 | 63.89 | ±5.58 | 42.7 | ±4.32 | 53.12 | ±4.83 | 46 | ±4.28 |
| AUC (h·µg/mL) | 0.57 | ±0.15 | 0.49 | ±0.11 | 20.55 | ±2.25 | 80.39 | ±3.76 | 118.41 | ±24.71 | 23.95 | ±3.26 | 18.88 | ±3.05 | 248.58 | ±21.17 |
| Tmax (h) | 1.8 | ±0.08 | 2.15 | ±0.11 | 4.54 | ±0.07 | 3.81 | ±0.14 | 3.9 | ±0.33 | 4.34 | ±0.35 | 3.74 | ±0.36 | 4.27 | ±0.19 |
| Cmax (µg/mL) | 0.11 | ±0.01 | 0.08 | ±0.01 | 0.71 | ±0.05 | 3.47 | ±0.32 | 3.55 | ±0.25 | 0.98 | ±0.02 | 0.8 | ±0.04 | 9.84 | ±0.39 |
| CL/F (mL/h/kg) | 87.33 | ±7.32 | 101.57 | ±10.08 | 2461.14 | ±258.80 | 623.26 | ±28.32 | 437.35 | ±72.92 | 2123.08 | ±266.31 | 2709.83 | ±384.11 | 202.49 | ±15.69 |
| Vd/F (mL/kg) | 82.49 | ±3.22 | 0.37 | ±0.04 | 73.189 | ±7332 | 14745 | ±2561 | 23567 | ±2142 | 52619 | ±2652 | 11655 | ±5254 | 22895 | ±4637 |

Note: α and β: exponential coefficients; T₁/₂₀₁: absorption rate constant; T₁/₂₁₀: central compartment elimination rate constant; T₁/₂α: half-life of α phase; T₁/₂β: half-life of β phase; AUC: area under the curve of plasma concentration-time; Tmax: the time point of maximum plasma concentration of the drug; Cmax: the maximum plasma concentration; CL/F: the apparent volume of the central compartment cleared of drug per unit time; Vd/F: Apparent volume of distribution based on the terminal elimination phase.

3.3. PK-PD Model Integration

The PK-PD parameters AUC₂₄h/MIC, AUC₂₄h/MPC, and T > MIC, T > MPC, integrating the PK-PD of acetylkitasamycin against S. suis, were 139.54 ± 5.30 h, 27.91 ± 1.06 h, and 45.54 h, 11.08 h, respectively. The inhibitory sigmoid Eₘₐₓ model flawless expressed the relationship between antimicrobial efficacy of acetylkitasamycin and the PK-PD parameter of AUC₂₄h/MIC ratio in PELF (Table 6). The parameters acquired were the values of N, E₀, PDₘₐₓ, EC₅₀ and AUC₂₄h/MIC, which represent different levels of antibacterial activity (Table 6).

3.4. Estimation and Assessment of Dose

According to the dosage equation, the optimal dose was calculated. CL/F was 202.49 ± 15.69 mL/h/kg, calculated by WinNonlin software. The MIC₅₀ was 1 µg/mL, and the fu was ignored. When E = 0 (bacteriostatic action), the AUC₂₄h/MIC was 57.65 h, the dosage calculated for bacteriostatic was 11.67 mg/kg. When E = −3 (bactericidal action), the AUC₂₄h/MIC was 163.56 h, the dosage calculated for bactericidal was 33.12 mg/kg. When E = −4 (eradication action), the AUC₂₄h/MIC was 407.12 h, the dosage calculated for eradication was 82.44 mg/kg.
Table 6. PK-PD integration parameters for acetylkitasamycin in PELF after intragastric administration at a dose of 50 mg/kg b.w. (n = 6).

| Time (h) | C_{vivo} (μg/mL) | (AUC)_{ex} (μg·h/mL) | E (logCFU/mL) | Calculated PD Target |
|----------|------------------|------------------------|---------------|----------------------|
| 0        | 0                | 0                      | 3.28          | E_0 = 3.28           |
| 0.5      | 0.22 ± 0.05      | 5.37 ± 1.25            | 0.31          | PD_{max} = 7.58      |
| 1        | 0.37 ± 0.08      | 8.96 ± 2.06            | -2.31         | N = 1.77 ± 0.34      |
| 2        | 0.71 ± 0.02      | 16.83 ± 0.63           | -3.63         | EC_{50} = 67.18 ± 8.32 |
| 4        | 0.88 ± 0.04      | 20.28 ± 2.07           | -4.30         |                      |
| 6        | 0.73 ± 0.04      | 17.93 ± 1.22           | -4.30         |                      |
| 8        | 0.51 ± 0.08      | 12.18 ± 2.1            | -2.93         |                      |
| 12       | 0.34 ± 0.12      | 8.12 ± 3.05            | -2.21         |                      |
| 24       | 0.22 ± 0.08      | 5.27 ± 1.92            | -1.47         |                      |
| 36       | 0.12 ± 0.05      | 2.77 ± 1.15            | 1.37          |                      |
| 48       | 0.07 ± 0.02      | 1.62 ± 0.49            | 2.31          |                      |
| 72       | 0.02 ± 0.009     | 0.55 ± 0.24            | 2.82          |                      |

MlxPlore software was used to simulate the effects of different doses (11.67, 33.12, 82.44 mg/kg) in vivo (Figure 5). On the basis of Figure 5, higher doses (33.12, 82.44 mg/kg) possessed bactericidal or eradication action during 0–12 h, but the bacterial regrowth occurred under the lower dose (11.67 mg/kg) treatment. Different dosing regimens (11.67 mg/kg every 12 h, 33.12 mg/kg every 12 h, 82.44 mg/kg every 12 h) were employed, simulating 3 days for treatment (Figure 5). At least 33.12 mg/kg every 12 h was sufficient to achieve bactericidal activity in PELF. Therefore, the dosing regimen of acetylkitasamycin for the treatment S. suis infection established as 33.12 mg/kg b.w. every 12 h for 3 days.

Figure 5. Model predictions of drug concentration (left) and bacterial growth (right) at different dose regimens with 12 h intervals by Mlxplore (adm1: Preventive dose, adm2: Therapeutic dose, adm3: Eradication dose).
4. Discussion

There is a substantial lack of preclinical and clinical PK data for acetylkitasamycin, and only the PK study in the ileum content has been studied [10]. The previous results indicated that the concentrations of every component of acetylkitasamycin in plasma were much lower than the MIC (1 µg/mL). On the contrary, the concentrations in PELF (9.84 µg/mL) were more than 10 times that of plasma. This result is the same as that of other macrolides; the concentrations of azithromycin and clarithromycin in foal PELF were more than 10 times that in foal plasma [8].

Recent evidence from this experiment and other studies (including animals and folks) have highlighted the importance of drug concentrations at the infection site in predicting the appropriate dosage for therapy. In addition to the macrolide drugs, other drugs such as doxycycline [37], moxifloxacin [38], linezolid [39] also have a higher concentrations in PELF. Therefore, the PK-PD study using the drug concentrations at the infection site can better reflect the actual clinical administration situation than using the drug concentrations in the plasma [8].

The $T_{\text{max}}$ values of the five main components $A_6A_7$, $A_5'$, $A_4A_5$, $A_1A_3$ and $A_{13}$ of acetylkitasamycin were 4.54 h, 3.81 h, 3.90 h, 4.34 h and 3.74 h, respectively. Analysis shows that there is no significant difference from the total $T_{\text{max}}$ value 4.27 h. Meanwhile, there was a significant difference between $C_{\text{max}}$ and AUC, due to the content of each component is different in pharmaceutical ingredients. Just like acetylkitasamycin, bitespiramycin has many components (Bitespiramycin I, II, III). After giving 80 mg/kg bitespiramycin to rats, the three main components reaching $T_{\text{max}}$ in plasma were 2.37 h, 2.69 h and 2.84 h, respectively, with no significant difference. However, there were significant differences in $C_{\text{max}}$ and AUC for the three main components [40].

To reveal the influence of every component in the total concentrations, weight coefficient ($w_j$) was introduced [41]. Every monomer composition of acetylkitasamycin in PELF concentrations was given its own weight coefficient, in order to calculate the total concentration ($C_T$). This was superior to simply summing the concentrations (enrofloxacin + ciprofloxacin) at the different time points [42]. Through the software WinNonlin 5.2, the values of $C_{\text{max}}$, $T_{\text{max}}$ and AUC were 9.84 µg/mL, 4.27 h and 248.58 h·µg/mL, respectively.

A very limited veterinary breakpoints has been properly established, and even most of testing laboratories continue routinely use human breakpoints [43]. Zafar’s research demonstrated that $S. \text{suis}$ resistance to macrolides increased steeply from 2002 to 2009, from 13% to 29.7% [44]. It is necessary to establish the breakpoint of acetylkitasamycin against $S. \text{suis}$. In this study, the wild-type cutoff value, one of the three cutoff values necessary to establish a breakpoint, was established as 16 µg/mL. There were 55 $S. \text{suis}$ strains, where MIC $\leq$ 16 µg/mL, and MIC$_50$ and MIC$_{90}$ were 1.21 µg/mL and 6.94 µg/mL, respectively.

The MIC of the clinical separation bacteria $S. \text{suis}$ HB1607 in MHB was not significantly greater than in PELF. It is worth noting that the total protein concentration in MHB used in this research was 3.78 g/L [45], and the corresponding concentrations in PELF were 0.25–0.62 g/L [46]. Therefore, the drug in PELF were perceived to be in free form when it comes to protein binding rates in PELF [33].

The PD target of $AUC_{24h}/\text{MIC} \geq 30$ was an indicator for the success of therapy and preventing emergence of resistance of macrolide [47]. In this study, the $AUC_{24h}/\text{MIC}$ obtained for bactericidal action in PELF was 137.71, which was much larger than 30. This may be because the concentrations in PELF were much higher than that in plasma. Zhanel [48] simulated the effect of azithromycin against $S. \text{pneumoniae}$ in vitro, and determined the PD target in serum, $AUC_{24h}/\text{MIC} \geq 36.7$, to be bactericidal. Schentag [49] believed that $AUC_{24h}/\text{MIC}$ was a good predictor of the effect of erythromycin in human plasma, with reported PD target $AUC_{24h}/\text{MIC} = 53$. Furthermore, some studies in the literature show that the parameter of AUC/MIC indicates that the effect is better than $T > \text{MIC}$, such as macrolides, azalides, ketolides and clindamycin [17,50].

In the past, dosing regimens for clinically used antimicrobials were generally developed on the basis of related PK data obtained in in vitro measurements of antibacterial
activity or the empirical clinical treatment, which are not based on solid PK-PD data. However, overuse and misuse of drugs is considered to be the primary factor that increases bacteria resistance in both humans and animals. PK-PD integration is regarded as a complimentary approach to PK-PD modeling for predicting the adequacy of the regimen in clinical subjects. According to the PK and PD data in this experiment, the dosages required to obtain different effects (bacteriostatic, bactericidal, eradication) were 11.67 mg/kg, 33.12 mg/kg, 82.44 mg/kg, respectively. After the professional software simulation, different dosages showed that 33.12 mg/kg treatment every 12 h for 3 days was sufficient to cure S. suis (HB1607). Therefore, the dosing regimen of acetylkitasamycin to treat S. suis was established as 33.12 mg/kg b.w. every 12 h for 3 days.

5. Limitations

According to the MIC determination of acetylkitasamycin against 110 S. suis strains, HB1607 with MIC close to MIC$_{50}$ and high pathogenicity was selected for the follow-up PD study, which was representative. However, considering the potential PD variability of each strain, this study cannot represent PD studies of acetylkitasamycin against all S. suis.

This study employed post-anesthesia sampling, a procedure necessary for animal welfare. Anesthesia may potentially affect the metabolism of drugs, but there are no reports of anesthetics affecting the metabolism of macrolides.

As shown in Table 5, there were large differences of drug concentrations in plasma and PELF, and only two components were detected in plasma with continuous concentrations. The authors believe that using plasma drug concentrations to calculate the administered dose would increase the dose of drug used during treatment and increase unnecessary risk. The purpose of this study was to determine the optimal dosing regimen of acetylkitasamycin for the treatment of S. suis infection, the PK data in target tissue will be more useful for the precise use of the drug to treat pneumonia. Although S. suis may cause systemic infections, the focus here was on pulmonary infections. Therefore, the drug concentrations in the PELF of lung tissue were used to calculate the dosing regimen.

6. Conclusions

The purpose of this study was to determine the adequacy regimen of acetylkitasamycin that could be effectively used to cure pigs infected with S. suis. The dosage determined was based on PK and PD data analysis. PD data were acquired from analysis of the static time kill curves obtained from ex vivo experiment. The inhibition $E_{\text{max}}$ equation was used to calculate the dosage. The dosing regimen of acetylkitasamycin to treat S. suis was established as 33.12 mg/kg b.w. every 12 h for 3 days.

**Author Contributions:** Conceptualization, A.H., F.M., W.Q., Z.Y., D.P. and H.H.; Data curation, F.M. and D.P.; Formal analysis, A.H., F.M. and Z.Y.; Funding acquisition, Z.Y. and D.P.; Investigation, Y.P. and G.C.; Methodology, A.H., F.M., S.X., Z.Y. and H.H.; Project administration, H.H.; Resources, L.H., Z.L., Z.Y., D.P. and H.H.; Supervision, L.H., S.X., Y.P., W.Q., G.C., Z.L., Z.Y., D.P. and H.H.; Writing—original draft, A.H. and F.M.; Writing—review & editing, H.H. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by grants from national key research and development program (2021YFD1800600/2016YFD0501302/2017YFD0501406), national natural science foundation of China (32172914/31772791), cooperative fund between Huazhong Agricultural University and Shenzhen Institute of agricultural genomics, Chinese Academy of Agricultural Sciences.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Animal Ethics Committee of Huazhong Agricultural University (HZAUSW 2015-016) and the Animal Care Center, Hubei Science and Technology Agency in China (SYXK 2013-0044).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is contained within the article.
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Feng, L.; Zhu, J.; Chang, H.; Gao, X.; Gao, C.; Wei, X.; Yuan, F.; Bei, W. The CodY regulator is essential for virulence in *Streptococcus suis* serotype 2. *Sci. Rep.* 2016, 6, 21241. [CrossRef] [PubMed]

2. Lun, Z.R.; Wang, Q.P.; Chen, X.G.; Li, A.X.; Zhu, X.Q. *Streptococcus suis*: An emerging zoonotic pathogen. *Lancet Infect. Dis.* 2007, 7, 201–209. [CrossRef]

3. Petrocchi-Rilo, M.; Martínez-Martínez, S.; Aguarón-Turrientes, I.; Roca-Martínez, E.; Gutiérrez-Martin, C.-B. Anatomical Site, Typing, Virulence Gene Profiling, Antimicrobial Susceptibility and Resistance Genes of *Streptococcus suis* Isolates Recovered from Pigs in Spain. *Antibiotics* 2021, 10, 707. [CrossRef] [PubMed]

4. Amsden, G.W. Advanced-generation macrolides: Tissue-directed antibiotics. *Int. J. Antimicrob. Agents* 2001, 18, 11–15. [CrossRef]

5. Omura, S. Microbial metabolites: 45 years of wandering, wondering and discovering. *Tetrahedron* 2011, 67, 6420–6459. [CrossRef]

6. Cerdá, R.; Giacoboni, G.I.; Xavier, J.A.; Landoni, P.L.S.F. In vitro antibiotic susceptibility of field isolates of *Mycoplasma synoviae* in Argentina. *Avian Dis.* 2002, 46, 215–218. [CrossRef]

7. Hardy, D.J.; Hensey, D.M.; Beyer, J.M.; Vojtko, C.; McDonald, E.J.; Fernandes, P.B. Comparative in vitro activities of new 14-, 15-, and 16-membered macrolides. *Antimicrob. Agents Chemother.* 1988, 32, 1710–1719. [CrossRef]

8. Suarez-Mier, G.; Giguère, S.; Lee, E.A. Pulmonary disposition of erythromycin, azithromycin, and clarithromycin in foals. *J. Vet. Pharmacol. Ther.* 2010, 30, 109–115. [CrossRef]

9. Mattoes, H.M.; Nightingale, C.H. Pharmacokinetics/Pharmacodynamics of Macrolides. In *Macrolide Antibiotics*; Birkhäuser: Basel, Switzerland, 2002.

10. Nan, J.; Hao, H.; Xie, S.; Pan, Y.; Yuan, Z. Pharmacokinetic and pharmacodynamic integration and modeling of acetylsalicylamycin in swine for *Clostridium perfringens*. *J. Vet. Pharmacol. Ther.* 2017, 40, 641. [CrossRef]

11. Yoshii, K.; Ikura, M.; Hirayama, M.; Toda, R.; Kawabata, Y. Physiologically-Based Pharmacokinetic and Pharmacodynamic Modeling for the Inhibition of Acetylcholinesterase by Acotiamide, A Novel Gastroprokinetic Agent for the Treatment of Functional Dyspepsia, in Rat Stomach. *Pharm. Res.* 2016, 33, 292–300. [CrossRef]

12. Rodríguez-Gascon, A.; Solinis, M.A.; Isla, A. The Role of PK/PD Analysis in the Development and Evaluation of Antimicrobials. *Pharmaceuticals* 2021, 13, 833. [CrossRef] [PubMed]

13. Asín-Prieto, E.; Rodríguez-Gascón, A.; Isla, A. Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. *J. Infect. Chemother.* 2021, 27, 319–329. [CrossRef] [PubMed]

14. Murthy, D.B.; Schmitt-Hoffmann, A. Pharmacokinetics and Pharmacodynamics of Cefotibiprole, an Anti-MRSA Cephalosporin with Broad-Spectrum Activity. *Clin. Pharmacokinet.* 2008, 47, 21–33. [CrossRef]

15. Jr, R.C.O.; Shorr, A.F. Rational dosing of antimicrobial agents: Pharmacokinetic and pharmacodynamic strategies. *Am. J. Health Syst. Pharm.* 2009, 66, 23–30.

16. Nielsen, E.L.; Cars, O.; Friberg, L.E. Pharmacokinetic/Pharmacodynamic (PK/PD) Indices of Antibiotics Predicted by a Semimechanistic PKPD Model: A Step toward Model-Based Dose Optimization. *Antimicrob. Agents Chemother.* 2011, 55, 4619–4630. [CrossRef] [PubMed]

17. Møller, J.K.; Singh, R.; Derendorf, H. Pharmacokinetic and pharmacodynamic implications in inhalable antimicrobial therapy. *Adv. Drug Deliv. Rev.* 2015, 85, 57–64. [CrossRef]

18. Ko, Y.H.; Song, P.H. Current Updates in Pharmacokinetics and Pharmacodynamics of Fluoroquinolones. *Korean J. Urogenit. Tract Infect. Inflamm.* 2015, 10, 1–6. [CrossRef]

19. Derksen, F.J.; Brown, C.M.; Sonea, I.; Darien, B.J.; Robinson, N.E. Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. *Equine Vet. J.* 2019, 110, 23–26. [CrossRef]

20. Xu, Z.; Huang, A.; Luo, X.; Zhang, P.; Huang, L.; Wang, X.; Mi, K.; Fang, S.; Huang, X.; Li, J.; et al. Exploration of Clinical Breakpoint of Danofloxacin for *Glaesserella parasuis* in Plasma and in PELF. *Antibiotics* 2021, 10, 808. [CrossRef]

21. Mouton, J.W.; Theuretzbacher, U.; Craig, W.A.; Tulkens, P.M.; Derendorf, H.; Cars, O. Tissue concentrations: Do we ever learn? *J. Antimicrob. Chemother.* 2008, 61, 235–237. [CrossRef] [PubMed]

22. Winther, L.; Baptiste, K.E.; Friis, C. Pharmacokinetics in pulmonary epithelial lining fluid and plasma of ampicillin and pivampicillin administered to horses. *Res. Vet. Sci.* 2012, 92, 111–115. [CrossRef] [PubMed]

23. Nourian, A.R.; Mills, P.C.; Pollitt, C.C. Development of an intra-lamellar microdialysis method for laminitis investigations in horses. *Vet. J.* 2010, 183, 22–26. [CrossRef] [PubMed]

24. Brunner, M.; Langer, O. Microdialysis versus other techniques for the clinical assessment of in vivo tissue drug distribution. *AAPS J.* 2006, 8, E263–E271. [CrossRef] [PubMed]

25. Kerdsin, A.; Dejsirilert, S.; Akeda, Y.; Sekizaki, T.; Hamada, S.; Gottschalk, M.; Oishi, K. Fifteen *Streptococcus suis* serotypes identified by multiplex PCR. *J. Med. Microbiol.* 2012, 61, 1669–1672. [CrossRef]

26. CLSI Development of In vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline. In *CLSI Document M37-A3*, 3rd ed.; Clinical and Laboratory Standards Institute: Philadelphia, PA, USA, 2007.

27. CLSI Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Four Informational Supplement. In *CLSI Document M100-S24*; Clinical and Laboratory Standards Institute: Philadelphia, PA, USA, 2014.
28. Lei, Z.X.; Liu, Q.Y.; Yang, S.K.; Yang, B.; Khaliq, H.; Li, K.; Ahmed, S.; Sajid, A.; Zhang, B.Z.; Chen, P.; et al. PK-PD Integration Modeling and Cutoff Value of Florfenicol against Streptococcus suis in Pigs. *Front. Pharmacol.* **2018**, *9*, 2. [CrossRef]

29. Wang, L.P.; Zhang, Y.S. Postantibiotic effects and postantibiotic sub-mic effects of tilmicosin, erythromycin and tiamulin on erythromycin-resistant *Streptococcus suis*. *Braz. J. Microbiol.* **2009**, *40*, 980–987. [CrossRef]

30. Zhou, Y.F.; Peng, H.M.; Bu, M.X.; Liu, Y.H.; Sun, J.; Liao, X.P. Pharmacodynamic Evaluation and PK/PD-Based Dose Prediction of Tulathromycin: A Potential New Indication for *Streptococcus suis* Infection. *Front. Pharmacol.* **2017**, *8*, 684. [CrossRef]

31. Zhang, L.; Li, Y.; Dai, K.; Wen, X.; Wu, R.; Huang, X.; Jin, J.; Xu, K.; Yan, Q.; Huang, Y.; et al. Establishment of a Successive Markerless Mutation System in *Haemophilus parasuis* through Natural Transformation. *PLoS ONE* **2015**, *10*, e0127393. [CrossRef]

32. Giguère, S.; Huang, R.; Malinski, T.J.; Dorr, P.M.; Tessman, R.K.; Somerville, B.A. Disposition of gamithromycin in plasma, pulmonary epithelial lining fluid, bronchoalveolar cells, and lung tissue in cattle. *Am. J. Vet. Res.* **2011**, *72*, 326–330. [CrossRef]

33. Kiem, S.; Schentag, J.J. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrob. Agents Chemother.* **2008**, *52*, 24–36. [CrossRef]

34. Pan, Y.H.; Zhang, H.Y.; Xi, C.L.; Huang, L.L.; Xie, S.Y.; Chen, D.M.; Tao, Y.F.; Liu, Z.L.; Yuan, Z.H. Simultaneous determination of multicomponent of acetykitasamycin and kitasamycin by LC-MS/MS in swine plasma and its application in a pharmacokinetic study. *Biomed. Chromatogr.* **2018**, *32*, e4268. [CrossRef] [PubMed]

35. Wang, J.; Hao, H.; Huang, L.; Liu, Z.; Chen, D.; Yuan, Z. Pharmacokinetic and Pharmacodynamic Integration and Modeling of Enrofloxacin in Swine for *Escherichia coli*. *Front. Microbiol.* **2017**, *8*, 36. [CrossRef] [PubMed]

36. Toutain, P.L.; Del Castillo, J.R.E.; Bousquet-Melou, A. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. *Res. Vet. Sci.* **2002**, *73*, 105–114. [CrossRef]

37. Womble, A.; Giguère, S.; Lee, E.A. Pharmacokinetics of oral doxycycline and concentrations in body fluids and bronchoalveolar cells of foals. *J. Vet. Pharmacol. Ther.* **2007**, *30*, 187–193. [CrossRef] [PubMed]

38. Gardner, S.Y.; Davis, J.L.; Jones, S.L.; Lafevers, D.H.; Hoskins, M.S.; Mcarver, E.M.; Papich, M.G. Moxifloxacin pharmacokinetics in horses and disposition into phagocytes after oral dosing. *J. Vet. Pharmacol. Ther.* **2004**, *27*, 57–60. [CrossRef]

39. Conte, J.E.; Golden, J.A.; Kipps, J.; Zurlinden, E. Intrapulmonary pharmacokinetics of linezolid. *Antimicrob. Agents Chemother.* **2002**, *46*, 1475–1480. [CrossRef]

40. Shi, X.; Zhong, D.; Su, N. Pharmacokinetics of a novel antibiotic bitespiramycin in rats. *Asian J. Drug Metab. Pharmacokinet.* **2003**, *3*, 134–137.

41. Liu, H.; Yang, J.; Du, F.; Gao, X.; Ma, X.; Huang, Y.; Xu, F.; Niu, W.; Wang, F.; Mao, Y. Absorption and Disposition of Ginsenosides after Oral Administration of *Panax notoginseng* Extract to Rats. *Drug Metab. Dispos.* **2009**, *37*, 2290–2298. [CrossRef] [PubMed]

42. Sang, K.; Hao, H.; Huang, L.; Wang, X.; Yuan, Z. Pharmacokinetic–Pharmacodynamic Modeling of Enrofloxacin Against *Escherichia coli* in Broilers. *Front. Vet. Sci.* **2015**, *2*, 80. [CrossRef]

43. Schwarz, S.; Böttner, A.; Goossens, L.; Hafez, H.M.; Hartmann, K.; Kaske, M.; Kehrenberg, C.; Kietzmann, M.; Krallmann, D.; Klein, G. A proposal of clinical breakpoints for amoxicillin applicable to porcine respiratory tract pathogens. *Vet. Microbiol.* **2008**, *126*, 178–188. [CrossRef]

44. Zafar, A.; Hasan, R.; Nizamuddin, S.; Mahmood, N.; Mukhtar, S.; Ali, F.; Morrissey, I.; Barker, K.; Torumkuney, D. Antibiotic susceptibility in *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Streptococcus pyogenes* in Pakistan: A review of results from the Survey of Antibiotic Resistance (SOAR) 2002–15. *J. Antimicrob. Chemother.* **2016**, *71*, i103–i109. [CrossRef] [PubMed]

45. Brentnall, C.; Cheng, Z.; McKellar, Q.; Lees, P. Pharmacodynamics of oxytetracycline administered alone and in combination with carprofen in calves. *Vet. Rec. J. Br. Vet. Assoc.* **2012**, *171*, 273. [CrossRef] [PubMed]

46. Hennig-Pauck, I.; Ganter, M.; Gerlach, G.F.; Rothkötter, H.J. Enzyme Activities, Protein Content and Cellular Variables in the Pulmonary Epithelial Lining Fluid in Selected Healthy Pigs. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **2001**, *48*, 631–639. [CrossRef] [PubMed]

47. Andes, D.; Anon, J.; Jacobs, M.R.; Craig, W.A. Application of pharmacokinetics and pharmacodynamics to antimicrobial therapy of respiratory tract infections. *Clin. Lab. Med.* **2004**, *24*, 477–502. [CrossRef]

48. Zhanel, G.G.; DeCorby, M.; Noreddin, A.; Mendoza, C.; Cumming, A.; Nichol, K.; Wierzbowski, A.; Hoban, D.J. Pharmacodynamic activity of azithromycin against macrolide-susceptible and-resistant *Streptococcus pneumoniae* simulating clinically achievable free serum, epithelial lining fluid and middle ear fluid concentrations. *J. Antimicrob. Chemother.* **2003**, *52*, 83–88. [CrossRef]

49. Schentag, J.J.; Klugman, K.P.; Yu, V.L.; Adelman, M.H.; Wilton, G.J.; Chiou, C.C.; Patel, M.; Lavin, B.; Paladino, J.A. *Streptococcus pneumoniae* bacteraemia: Pharmacodynamic correlations with outcome and macrolide resistance—A controlled study. *Int. J. Antimicrob. Agents* **2007**, *30*, 264–269. [CrossRef]

50. Carral, N.; Lukas, J.C.; Oteo, I.; Suarez, E. Impact of poor compliance with levofloxacin and moxifloxacin on respiratory tract infection antimicrobial efficacy: A pharmacokinetic/pharmacodynamic simulation study. *Int. J. Antimicrob. Agents* **2015**, *45*, 79–83. [CrossRef]