Susceptibility Breakpoint for Enrofloxacin against Swine *Salmonella* spp.

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Susceptibility breakpoints are crucial for prudent use of antimicrobials. This study has developed the first susceptibility breakpoint (MIC ≤ 0.25 µg/ml) for enrofloxacin against swine *Salmonella* spp. based on wild-type cutoff (CO_{WT}) and pharmacokinetic-pharmacodynamic (PK-PD) cutoff (CO_{PD}) values, consequently providing a criterion for susceptibility testing and clinical usage of enrofloxacin.

*S*almonella spp. are leading zoonosis and food-borne pathogens. Approximately 10 to 20% of all human cases of salmonellosis in the European Union may be the result of contact with pigs and consumption of pig meat (1). Enrofloxacin, an FDA- and China-approved fluoroquinolone member, has been used broadly for treatment of swine disease caused by Gram-positive and -negative bacteria. For guiding susceptibility testing and clinical drug usage, the Clinical and Laboratory Standards Institute (CLSI) has developed a susceptibility breakpoint for enrofloxacin in swine for respiratory disease only (2). Before the present study, no breakpoint for enrofloxacin had been established for swine disease caused by enteric bacteria, such as *Salmonella*.

In the present study, 214 swine *Salmonella* isolates were obtained from five representative districts (Henan, Hubei, Zhejiang, Anhui, and Shanghai) in China during the years 2003 to 2010. The MICs of enrofloxacin to these swine *Salmonella* isolates were determined by agar dilution susceptibility testing according to the CLSI M31-A3 guidance (2). Primary MIC distribution was subjected to statistical goodness-of-fit tests and nonlinear least-squares regressions by following the procedure elaborated in a previous study (3). A wild-type cutoff (CO_{WT}) was developed based on the fitted MIC distribution by following CLSI M37-A3 guidance and some previous methods (3–5).

As shown in the primitive enrofloxacin MIC distribution in Fig. 1A, MICs for enrofloxacin against 214 *Salmonella* isolates were in the range of approximately 0.125 to 8 µg/ml. The percentages at each MIC (0.125, 0.25, 0.5, 1, 2, 4, and 8 µg/ml) were 6.1%, 0.4%, 14.5%, 15%, 43.5%, 5.6%, and 15%, respectively. The standard goodness-of-fit tests demonstrated that primitive MIC distribution did not match a normal distribution, because a bimodal distribution was observed at MICs of 2 µg/ml and 8 µg/ml. To obtain unimodal MIC distribution, the 32 *Salmonella* isolates with a MIC of 8 µg/ml were consequently removed. The other 182 swine *Salmonella* isolates were subsequently subjected to nonlinear least-squares regressions and goodness-of-fit tests. The best fit for the unimodal population was found when presumed MIC distribution was defined as being between 0.125 µg/ml and 4.0 µg/ml. In the fitted unimodal MIC distribution (see Fig. 1B), the number of isolates estimated by nonlinear least-squares regression (183 isolates) was closest to the true number of isolates (182 isolates). Of the estimated number of *Salmonella* strains (183 isolates), more than 95% had enrofloxacin MICs in the range of
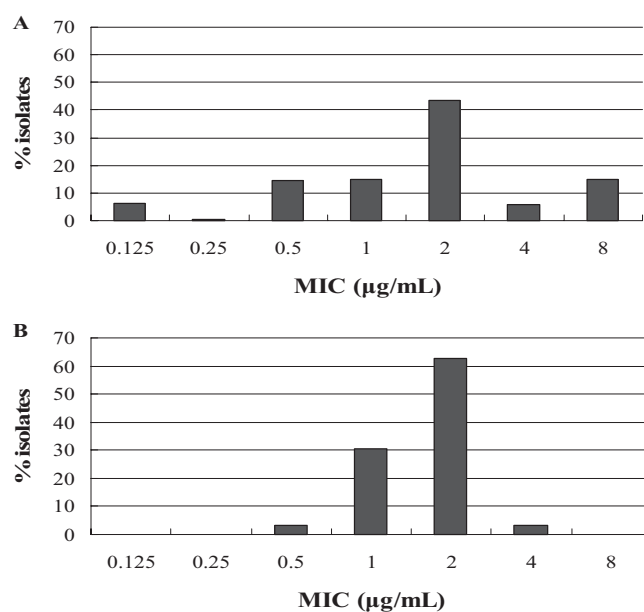

FIG 1 The MIC distribution for enrofloxacin against *Salmonella* isolates. (A) Primary MIC distribution of 214 *Salmonella* isolates; (B) fitted MIC distribution of the estimated 183 *Salmonella* isolates after standard goodness-of-fit tests and nonlinear least-squares regression.

approximately 0.5 to 2 µg/ml. After NORMINV function and NORMDIST test in Microsoft Excel, the CO_{WT} was defined as 2.0 µg/ml. The CO_{WT} in our study was slightly higher than susceptibility MIC breakpoints for ciprofloxacin against human *Enterobacteriaceae* recommended by CLSI M100-S20 (MIC ≤ 1 µg/ml)

TABLE 1 Plasma pharmacokinetic parameters after single-dose intramuscular administration of enrofloxacin (2.5 mg/kg of body weight) to piglets ($n = 12$)

Pharmacokinetic parameter[a]	Unit	Value ± SD
A	µg/ml	2.19 ± 0.18
Ke	1liter/h	0.13 ± 0.03
Ka	1liter/h	4.97 ± 2.47
Lag time	h	0.02 ± 0.08
$t_{1/2}$(Ka)	h	0.14 ± 0.03
$t_{1/2}$(Kb)	h	5.17 ± 1.65
t (peak)	h	0.74 ± 0.11
C_{max}	µg/ml	0.74 ± 0.38
AUC	(µg/ml) · h	15.87 ± 3.39
CL/F(s)	ml/kg/h/(µg/ml)	0.33 ± 0.12
V/F(C)	(ml/kg)/(µg/ml)	2.30 ± 0.30
Protein binding	%	41.4–56.6

[a] A, zero time intercept for α phase; Ke, elimination rate constant; Ka, absorption rate constant; $t_{1/2}$(Ka), half-life of absorption; $t_{1/2}$(Kb), half-life of elimination; t (peak), peak time; C_{max}, peak drug concentration; AUC, area under the drug concentration-time curves; CL/F(s), total body clearance; V/F(C), volume of distribution at steady state.

TABLE 2 Accumulated probability of attaining the target AUC/MIC ratio at specific MIC breakpoints

Target AUC/MIC ratio	Accumulated target attainment probability (%) at each MIC (µg/ml)						
	0.125	0.25	0.5	1	2	4	8
50	100.00	100.00	63.90	35.82	6.47	7.58	12.85
60	100.00	100.00	49.13	29.86	4.94	5.70	9.42
70	100.00	100.00	38.89	25.43	3.96	4.67	7.19
80	100.00	100.00	33.46	22.08	3.26	4.05	5.47
90	100.00	99.99	29.22	19.51	2.80	3.48	4.42
100	100.00	99.94	25.47	17.11	2.40	3.10	3.64
110	100.00	99.74	22.33	15.21	2.08	2.70	3.01
125	100.00	98.43	18.97	13.03	1.79	2.40	2.17

and the traditional ciprofloxacin breakpoint against *Salmonella* (MIC ≤ 1 µg/ml) used in the 2002 to 2010 NARMS reports (6, 7). The higher CO_{WT} in the present study may due to the different backgrounds of strains isolated from different hosts and different geographical areas.

After single-dose intramuscular administration of enrofloxacin (2.5 mg/kg of body weight) to 12 piglets, concentrations of enrofloxacin in plasma were determined by high-performance liquid chromatography (HPLC) according to the method established in our lab (8). The pharmacokinetic (PK) parameters were calculated using 3p97 software. Based on human clinical experience, an area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC ratio) value of ≥100 was assumed as pharmacokinetic-pharmacodynamic (PK-PD) target attainment for fluoroquinolones against Gram-negative organisms (9). A 10,000-subject Monte Carlo simulation was constructed using Crystal Ball Professional version 7.2.2 software. The PK-PD susceptibility cutoff (CO_{PD}) was defined as the MIC at which the probability of target attainment (PTA) was ≥90% (4).

Our results were in agreement with previous studies which indicated that ciprofloxacin levels (an active metabolite of enrofloxacin) were too low in the plasma of pigs and enrofloxacin could be served as the marker for PK calculation (10, 11). Based on the concentration-time curve of plasma enrofloxacin, a series of pharmacokinetic parameters for the 12 piglets were derived from a one-compartment model (see Table 1). After intramuscular administration of enrofloxacin, the peak drug concentration (C_{max}) and AUC were 0.74 ± 0.38 µg/ml and 15.87 ± 3.39 µg · h/ml, respectively. The probability of achieving various AUC/MIC ratios at each MIC value after a 10,000-pig Monte Carlo simulation is presented in Table 2. At a MIC value of ≤0.25 µg/ml, the probabilities of achieving AUC/MIC values of ≥100 were higher than 90%. Therefore, the CO_{PD} of enrofloxacin against swine *Salmonella* was defined as a MIC value of ≤0.25 µg/ml.

The CO_{PD} in our study (MIC ≤ 0.25 µg/ml) was lower than the PK-PD breakpoint for ciprofloxacin recommended by EUCAST (MIC ≤ 0.5 µg/ml) and the susceptibility breakpoint for enro-

floxacin against dog *Enterobacteriaceae* (MIC ≤ 0.5 µg/ml) recommended by CLSI M31-A3 (6, 12). The lower PK-PD breakpoint in our study may be due to the lower dose of drug administration to pigs, because previous studies concluded that the dose of drug administration may affect the PK-PD breakpoint (11, 13, 14). The PK-PD cutoff developed in our study should be more conservative, because it was generated based on the lowest approved dosage regimen for enrofloxacin (15). Based on PK-PD models with the Monte Carlo simulation, a recent study established a ciprofloxacin breakpoint for Gram-negative aerobic bacteria (MIC ≤ 0.125 µg/ml) which was also much lower than the CLSI breakpoint and EUCAST breakpoint (16). Both of the previous studies and our study suggested that CLSI may need to revise the breakpoint for some Gram-negative bacteria (16, 17). Coincidently, a new susceptibility breakpoint for ciprofloxacin against *Salmonella* (MIC < 0.125 µg/ml) was used in a 2011 NARMS report (7). The discrepancy of susceptibility breakpoint may be due to drug specificity and geographical differences.

Conclusively, our study is unique in the sense that it has established a novel enrofloxacin susceptibility breakpoint against swine *Salmonella* spp. based on CO_{WT} (MIC ≤ 2 µg/ml) and CO_{PD} (MIC ≤ 0.25 µg/ml). Since the PK-PD cutoff provided greater value for setting the breakpoint than the wild-type cutoff did (15), the CO_{PD} breakpoint (MIC ≤ 0.25 µg/ml) was finally selected as the optimum enrofloxacin susceptibility breakpoint for swine *Salmonella*. Although further clinical studies are necessary for confirming our findings, our work, to some extent, could provide a criterion for enrofloxacin susceptibility testing and improve prudent use of enrofloxacin for public health.

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