Measurement of plasma protein binding

The average unbound fraction (*f*u) of polymyxin B in the plasma of neutropenic mice with lung infections was determined using ultracentrifugation as described previously (1). In brief, aliquots of the samples were incubated at 37.8^{o}C for 30 min prior to ultracentrifugation at 279,000 × *g* for 4 h (OptimaTM MAX-TL ultracentrifuge, fitted with a TLA-100 fixed-angle rotor; Beckman Coulter, Inc., Indianapolis, IN, USA). The supernatant (50 µL) was removed from each of the two replicate samples and as was the resuspended content of another replicate sample. The supernatant and resuspended plasma samples were diluted with an equal volume of drug-free mouse plasma and pH 7.4 phosphate buffer saline (PBS), respectively. The concentration of polymyxin B in the re-suspended plasma and supernatant samples were determined by a validated LC-MS/MS assay with minor modifications (2). The calibration curves ranged from 0.10 mg/L to 10.0 mg/L and were prepared in equal mixture of blank plasma and pH 7.4 PBS. The limit of quantification was 0.10 mg/L.

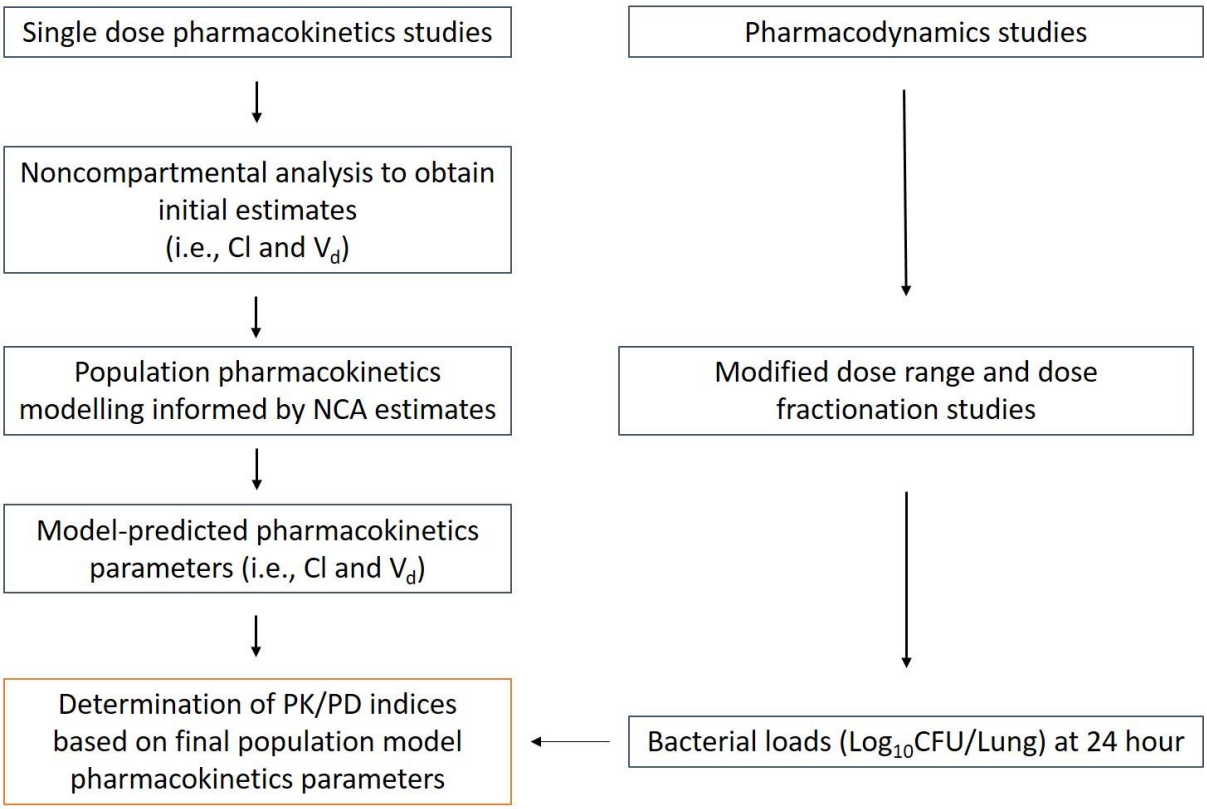

Figure S1. Flow chart of the post-processing pharmacokinetics/pharmacodynamics (PK/PD) data analysis.

References

1. Cheah S-E, Wang J, Turnidge JD, Li J, Nation RL. 2015. New pharmacokinetic/pharmacodynamic studies of systemically administered colistin against Pseudomonas aeruginosa and Acinetobacter baumannii in mouse thigh and lung infection models: smaller response in lung infection. J Antimicrob Chemother **70:**3291-3297.
2. Cheah S-E, Bulitta JB, Li J, Nation RL. 2014. Development and validation of a liquid chromatography–mass spectrometry assay for polymyxin B in bacterial growth media. J Pharm Biomed Anal **92:**177-182.