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

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**Objectives:** This study investigated the exposure–response relationships between unbound colistin in plasma and antibacterial activity in mouse thigh and lung infections.

**Methods:** Dose fractionation studies (subcutaneous colistin sulphate at 1.25–160 mg/kg/day) were conducted in neutropenic mice in which infection (three strains of *Pseudomonas aeruginosa* and three strains of *Acinetobacter baumannii*) had been produced by intramuscular thigh injection or aerosol lung delivery. Bacterial burden was measured at 24 h after initiation of colistin treatment. Plasma protein binding was measured by rapid equilibrium dialysis and ultracentrifugation. The inhibitory sigmoid dose–effect model and non-linear least squares regression were employed to determine the relationship between exposure to unbound colistin and efficacy.

**Results:** Plasma binding of colistin was constant over the concentration range ≏2–50 mg/L. The average ± SD percentage bound for all concentrations was 92.9 ± 3.3% by ultracentrifugation and 90.4 ± 1.1% by equilibrium dialysis. In the thigh model, across all six strains the antibacterial effect of colistin was well correlated with fAUC/MIC (R² = 0.82–0.94 for *P. aeruginosa* and R² = 0.84–0.95 for *A. baumannii*). Target values of fAUC/MIC for 2 log₁₀ kill were 7.4–13.7 for *P. aeruginosa* and 7.4–17.6 for *A. baumannii*. In the lung model, for only two strains of *P. aeruginosa* and one strain of *A. baumannii* was it possible to achieve 2 log₁₀ kill (fAUC/MIC target values 36.8–105), even at the highest colistin dose tolerated by mice. This dose was not able to achieve bacteriostasis for the other two strains of *A. baumannii*.

**Conclusions:** Colistin was substantially less effective in lung infection. The pharmacokinetic/pharmacodynamic target values will assist in the design of optimized dosage regimens.

**Introduction**

Over the last several years colistin (polymyxin E) and polymyxin B have increasingly been used for the treatment of Gram-negative infections that are resistant to most other antibiotics. After their discovery, the polymyxins were not subjected to the drug development and regulatory approval processes used today. As a result, the pharmacological information needed to guide their optimal use in patients was not generated prior to their introduction into the clinic.¹ Over the last 15 years a number of studies have been conducted to generate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the polymyxins and the relationship between their PK and PD.²,³

Colistin and polymyxin B display concentration-dependent killing of Gram-negative bacteria.⁴–¹¹ In dose-fractionation studies conducted against *Pseudomonas aeruginosa* in in vitro PK/PD models, the ratio of the area under the unbound concentration–time curve to the MIC (fAUC/MIC) has been shown to be the PK/PD index that best predicts bacterial killing by both colistin and polymyxin B.⁶,¹¹ Similarly, dose-fractionation studies of colistin against *P. aeruginosa* and *Acinetobacter baumannii* in mouse infection models have revealed that AUC/MIC (i.e. for total drug in plasma)¹²–¹⁴ and fAUC/MIC (i.e. for unbound drug in plasma)¹⁵,¹⁶ correlate with bacterial killing in vivo.

In our previous mouse infection studies,¹⁵,¹⁶ the determination of fAUC/MIC values for various magnitudes of bacterial killing
relied on measurements of colistin protein binding in the plasma of infected neutropenic mice. Those protein-binding experiments were conducted in Plexiglas® equilibrium dialysis cells and suggested concentration-dependent plasma protein binding of colistin. In view of the physicochemical properties of colistin and its potential for adsorption to surfaces of laboratory ware, we have more recently measured colistin plasma protein binding by equilibrium dialysis in Teflon® cells and by ultracentrifugation, techniques that are particularly well suited to molecules that are prone to adsorption on surfaces. In the earlier mouse lung infection studies on colistin the infection was initiated by instillation of a bacterial suspension into the nares of the animals. More recently, in projects with a range of different antibiotics, we have moved to inoculation via intratracheal inoculation as reported by other investigators. This more direct introduction of bacteria into the lungs provides a reproducible infection that may be more representative of pneumonia. We report here the use of this mouse model for dose-fractionation studies of colistin against lung infections caused by P. aeruginosa and A. baumannii. Also included are results of our new plasma protein binding studies conducted by equilibrium dialysis in Teflon® cells and ultracentrifugation, and the application of these results to the determination of fAUC/MIC values for various magnitudes of bacterial killing in the previous thigh infection model and the improved lung infection model.

**Materials and methods**

**Chemicals and bacterial strains**

Colistin sulphate (Lot 081M1526V; ≥15000 U/mg) was purchased from Sigma-Aldrich (St Louis, MO, USA) and solutions were prepared for administration to mice as reported previously. Three strains of P. aeruginosa (ATCC 27853 (MIC 1 mg/L), PAO1 (1 mg/L) and a clinical MDR mucoid isolate, 19056 (0.5 mg/L)) and three strains of A. baumannii (ATCC 19606 (1 mg/L) and two MDR clinical isolates, 248-01-C248 (1 mg/L) and N-16870.213 (0.5 mg/L)) were employed and subcultured prior to experiments, as described previously. MICs were determined by broth microdilution in the absence of polysorbate. 8

**Neutropenic murine thigh and lung infection models**

Animal experiments were approved by the institutional animal ethics committee (approval number VCPA.2008.09) and animals were maintained in accordance with the criteria of the Australian code of practice for the care and use of animals for scientific purposes. Eight-week-old, specific-pathogen-free, female Swiss mice (24 – 30 g) were obtained from Monash Animal Services (Clayton, Victoria, Australia) and were fed, housed and rendered neutropenic by administration of cyclophosphamide, as described previously. Lung infection was induced by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and rested in the supine position against a restraining board that was angled at 60 – 70° from the horizontal. Each mouse was inoculated with 25 μL of bacterial suspension in saline (≥106 bacterial cells in early logarithmic growth phase) sprayed directly into the trachea above the carina using a MicroSprayer® Aerosolizer (model IA-1C; Penn-Century, Philadelphia, PA, USA). After discharge of the bacterial aerosol, mice were placed upright on a warm pad for recovery from anaesthesia. We also undertook additional dose-fractionation studies in the mouse thigh infection model to validate and supplement data reported previously. Colistin treatment (subcutaneous injection of dose-fractionated

**PK of colistin in neutropenic infected mice**

Single-dose PK studies (10, 20 and 40 mg/kg colistin sulphate) were performed in neutropenic infected mice, as described previously. These were undertaken because of the elapsed time since the original studies and the recent availability of a more sensitive assay. The original PK studies employed an HPLC assay to quantify colistin in plasma, while in the present studies we used a more sensitive LC–MS/MS assay, modified from a previously reported method. The calibration range was from 0.1 mg/L (limit of quantification) to 20 mg/L; samples above the latter concentration were appropriately diluted prior to analysis. Analysis of independently prepared quality control samples indicated that the reproducibility (coefficients of variation ≤5.83%) and accuracy (deviations between measured and nominal concentrations ≤14.6%) of the assay were acceptable. The plasma concentration versus time data were subjected to non-compartmental analysis (Phoenix WinNonlin software, version 6.3; Pharsight Corporation) to obtain the PK parameters.

**Plasma protein binding**

The protein binding of colistin in pooled plasma collected from infected neutropenic mice was determined by ultracentrifugation and by equilibrium dialysis in Teflon® dialysis cells. Drug-free plasma was spiked to achieve multiple colistin concentrations in the range 2 – 50 mg/L, corresponding to the relevant range of total plasma concentrations in the PK studies and the concentrations associated with bacterial killing in the PD studies. For ultracentrifugation, aliquots (200 μL) of each sample were incubated for 30 min at 37 °C then ultracentrifuged at 279000 g for 4 h at 37 °C (OptimaTM MAX-TL ultracentrifuge, fitted with a TLA-100 fixed-angle rotor; Beckman Coulter, Inc., Indianapolis, IN, USA). An aliquot (50 μL) of protein-free supernatant was mixed with an equal volume of drug-free (‘blank’) plasma while re-suspended plasma samples were mixed with an equal volume of pH 7.4 buffer. The concentrations of colistin in the supernatant samples were measured with an equal volume of drug-free (‘blank’) plasma while re-suspended plasma samples were mixed with an equal volume of pH 7.4 buffer (i.e. the same composition as the unknowns). The protein binding of colistin in plasma of infected neutropenic mice was also measured using equilibrium dialysis. Plasma (1 mL) was dialysed in Teflon® dialysis cells (Dianorm®; Harvard Apparatus, Holliston, MA, USA) across a semipermeable membrane (Spectra/Por®-2, molecular weight cut-off 12 000 – 14 000 Da) against an equal volume of pH 7.4 isotonic phosphate buffer (0.067 M) at 37 °C for 4 h (a time sufficient to reach equilibrium). At that time, the plasma or buffer in the respective compartments was expelled (through the exit port by a slow flow of air delivered via another port, in accordance with the user manual for the Dianorm® system) into pre-weighted tubes, the tubes were re-weighed and the contents mixed with an equal volume of buffer or blank plasma, respectively. That is, the post-equilibration plasma and buffer samples were not removed using pipette tips. Colistin concentrations in the
resulting diluted plasma and buffer samples were quantified using an LC–MS/MS assay as described above, and used to determine protein binding.

**Data analysis**

The average plasma unbound fraction from the ultracentrifugation and equilibrium dialysis studies across the concentration range \(2–50\text{ mg/L}\) was used to determine the time-course of unbound plasma colistin concentration in the single-dose PK study. The superposition principle was applied to the unbound plasma concentration–time data from the single-dose studies to obtain the corresponding time-course over 24 h, for multiple administrations of the respective dose at the various dosage intervals used in the PK/PD studies.\(^{26}\) The PK for dose levels that were not examined in the single-dose study were determined by interpolation from dose levels for which PK were directly measured and extrapolation for dose levels below the lowest dose level examined, as described previously.\(^{27}\) From each of these multiple-dose profiles the PK/PD indices for unbound drug (i.e., \(\text{AUC}/\text{MIC}, \text{AUC}_{\text{max}}/\text{MIC}\) and \(\text{FT}_{\text{MIC}}\)) were determined. Subsequently, each of these indices was subjected to PK/PD analysis by use of the inhibitory sigmoid dose–effect model and non-linear least squares regression analysis, as described previously.\(^{15}\)

**Results and discussion**

The percentage of colistin bound in plasma of infected neutropenic mice was independent of plasma concentration over the range \(2–50\text{ mg/L}\), when assessed by both ultracentrifugation and equilibrium dialysis in low-sorption cells (Figure 1). The average \(\pm\) SD percentage bound for all plasma colistin concentrations presented in the figure was 92.9 \(\pm\) 3.3\% when binding was measured by ultracentrifugation and 90.4 \(\pm\) 1.1\% by equilibrium dialysis; the average of the two methods was 91.6\%. Thus, the average plasma unbound fraction for colistin was 0.084 (Figure 1). These results differ from the concentration-dependent plasma binding reported previously for neutropenic infected mice.\(^{15}\) That earlier study involved equilibrium dialysis in Plexiglas\textsuperscript{®} cells over 21 h. In the present study, two independent methods were used, namely ultracentrifugation and equilibrium dialysis in Teflon\textsuperscript{®} cells having a high dialysis membrane surface area-to-volume ratio, allowing equilibrium to be reached in 4 h. Both methods used in the present study are well suited to the determination of plasma binding of drugs that exhibit extensive non-specific binding to laboratory equipment.\(^{18–21}\) The excellent agreement of the plasma binding results of these two methods (Figure 1) is reassuring. Recently, we have also determined the binding of colistin in plasma of 66 critically ill patients (and healthy humans) to be \(\sim 50\%\) (unbound fraction \(\sim 0.5\)). That is, the unbound fraction is \(\sim 6\)-fold higher in humans than in mice. The detailed results of those clinical studies form a component of an extensive population PK/PD analysis (R. L. Nation, S. M. Garonzik, J. Li, V. Thamlakitkul, E. J. Giamarellos-Bourboulis, D. L. Paterson, J. D. Turnidge, A. Forrest and F. P. Silveira, unpublished results).

The total plasma colistin concentration versus time profiles from the PK studies conducted in the present investigation are shown in Figure 2. These profiles are in very good agreement with those reported previously.\(^{15}\) The derived PK parameters from the profiles in Figure 2 are presented in Table 1. The total plasma colistin concentrations in the figure were used along with the average plasma unbound fraction discussed above (i.e., 0.084) to generate the corresponding time-course profiles for unbound colistin. The latter were used to determine the \(\text{AUC}/\text{MIC}, \text{AUC}_{\text{max}}/\text{MIC}\) and \(\text{FT}_{\text{MIC}}\) values for the various dosage regimens used in the PK/PD study. Across the six strains of \(P.\ aeruginosa\) and \(A.\ baumannii\), \(\text{AUC}/\text{MIC}\) provided an overall superior description of the PD data (lowest \(R^2\) values for \(\text{AUC}/\text{MIC}, \text{AUC}_{\text{max}}/\text{MIC}\) and \(\text{FT}_{\text{MIC}}\) were 0.82, 0.74 and 0.51, respectively), as has been reported previously by us and others for colistin and polymyxin B in \(\text{in vitro}\) and \(\text{in vivo}\) preclinical models.\(^{6,11–16}\)

The relationships between the antibacterial effect of colistin and \(\text{AUC}/\text{MIC}\) for \(P.\ aeruginosa\) ATCC 27853 and \(A.\ baumannii\) ATCC 19606 in the thigh infection model are shown in Figure 3. These plots comprise the data from the current and corresponding previous\(^{15,16}\) studies. The data were combined because across the range of daily doses and fractionated regimens at a given daily dose there was good agreement in the level of effect between

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**Figure 1.** Unbound fraction of colistin in plasma of infected neutropenic mice over the plasma colistin concentration range \(2–50\text{ mg/L}\). Plasma binding was measured using either ultracentrifugation or equilibrium dialysis.

**Figure 2.** Total plasma colistin concentrations versus time after administration of single subcutaneous doses of 10, 20 or 40 mg/kg colistin (sulphate) in neutropenic infected mice. Each symbol represents the mean \(\pm\) SD for four mice.
the respective earlier and current studies. The fits in Figure 3 and those for the other two strains of each bacterial species were well described by the inhibitory sigmoid dose–effect model ($R^2 = 0.82–0.94$ for P. aeruginosa and $R^2 = 0.84–0.95$ for A.baumannii) and the PK/PD parameters were estimated with good precision (Table 2). The values of fAUC/MIC for stasis and 1 and 2 log10 reductions in bacterial counts are presented in Table 3. All target values in the table were determined by measurement of MIC using broth microdilution without addition of polysorbate 80. For both species, there was an ~2-fold range in the fAUC/MIC values associated with a 2 log10 reduction in bacterial numbers (fAUC/MIC range of 7.4–13.7 for P. aeruginosa and 7.4–17.6 for A. baumannii). The target values of fAUC/MIC for various magnitudes of antibacterial effect are lower, particularly for the higher levels of bacterial kill, than those reported previously for P. aeruginosa and A. baumannii in the thigh infection model. This arose because of the difference in plasma protein binding results between the former study, where concentration-dependent plasma unbound fraction of colistin was observed, and the current study, where binding measured by two independent methods was consistent across the methods and indicated constancy of binding over the relevant concentration range (Figure 1).

In the lung infection model, colistin was substantially less effective in killing the bacteria. The relationship between bacterial burden in lungs and fAUC/MIC for P. aeruginosa ATCC 27853 is shown in Figure 4(a); the profiles for the other two strains of this species were very similar in form. For all three strains of P. aeruginosa it was not possible to achieve a maximal reduction in bacterial numbers. This was due to the inability of animals to tolerate colistin doses ~60 mg/kg administered at a given time and regimens with cumulative daily doses ~120–160 mg/kg. Due to the inability to observe a maximal effect, it was not possible to derive precise estimates from the fitted inhibitory sigmoid dose–effect model of maximal effect, the value of fAUC/MIC required to elicit 50% of maximal effect and the Hill coefficient. However, the data available for colistin exposures up to the highest tolerated doses were sufficient to produce satisfactory curve fits (Figure 4(a) and the data for the other two strains), allowing estimation of the fAUC/MIC values associated with stasis and 1 and 2 log10 bacterial kill (Table 3). The target values for systemically administered colistin against P. aeruginosa PAO1 in the current study compare well with those from another study conducted by others with the same strain in neutropenic mice with lung infection initiated by intratracheal inoculation. In the latter study, the fAUC/MIC for stasis was ~14 [calculated from the data for total plasma colistin concentrations (i.e. AUC/MIC) presented in Table 4 of that paper and applying a plasma unbound fraction of 0.084 as determined in the present study]. By way of comparison, against P. aeruginosa PAO1 in the study reported here the colistin fAUC/MIC for stasis was very similar at 15.2.

In regard to A. baumannii in the lung infection model, there was only one strain (248-01-C248) where bacterial killing was observed. The nature of the relationship between the number of viable bacteria per lung and fAUC/MIC was very similar to that shown for the P. aeruginosa strains, as exemplified for one such strain in Figure 4(a). For A. baumannii strain 248-01-C248, at the highest tolerated colistin dosage regimen there was ~2 log10 of bacterial killing, but the maximal effect was not

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**Table 1.** PK parameters for total plasma concentrations of colistin following subcutaneous administration of single doses (10 to 40 mg/kg colistin sulphate) in neutropenic infected mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result for a single subcutaneous dose of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>11.1</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>60</td>
</tr>
<tr>
<td>VIF (L/kg)</td>
<td>0.530</td>
</tr>
<tr>
<td>CL/F (mL/min/kg)</td>
<td>5.08</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>72.4</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ and $T_{\text{max}}$ are maximum plasma concentration and time of its occurrence, VIF and CL/F are the apparent volume of distribution and clearance conditioned on the unknown bioavailability (F) and $t_{1/2}$ is terminal half-life.
able to be determined. With the other two \textit{A. baumannii} strains, there was no decrease in bacterial numbers per lung across a wide range of \(\text{fAUC/MIC}\) values, up to the exposure level generated by the highest tolerated dosage regimen of colistin (Figure 4b). It was not appropriate to explore higher colistin dosage regimens. Thus, for \textit{A. baumannii} strains ATCC 19606 and N-16870.213 it was not possible to estimate \(\text{fAUC/MIC}\) values for stasis and 1 and 2 log10 reductions in bacterial numbers (Table 3).

### Table 2. PK/PD model parameter estimates for the \(\text{fAUC/MIC}\) index of colistin against all three strains of \textit{P. aeruginosa} and \textit{A. baumannii} in the thigh infection model

<table>
<thead>
<tr>
<th>Species/strain</th>
<th>(E_{\text{max}}) (log10 cfu/thigh)</th>
<th>(E_0) (log10 cfu/thigh)</th>
<th>EC(_{50})</th>
<th>(\gamma)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{P. aeruginosa} ATCC 27853</td>
<td>6.13 (8.48)</td>
<td>8.61 (2.49)</td>
<td>11.0 (9.24)</td>
<td>3.02 (21.9)</td>
<td>0.82</td>
</tr>
<tr>
<td>PAO1</td>
<td>4.97 (3.64)</td>
<td>8.42 (1.48)</td>
<td>6.11 (2.14)</td>
<td>10.0 (25.3)</td>
<td>0.94</td>
</tr>
<tr>
<td>19056</td>
<td>6.84 (11.2)</td>
<td>8.70 (2.99)</td>
<td>9.28 (13.1)</td>
<td>2.16 (24.2)</td>
<td>0.90</td>
</tr>
<tr>
<td>\textit{A. baumannii} ATCC 19606</td>
<td>3.78 (9.13)</td>
<td>7.46 (2.89)</td>
<td>2.97 (16.4)</td>
<td>1.31 (21.9)</td>
<td>0.84</td>
</tr>
<tr>
<td>248-01-C.248</td>
<td>4.26 (7.27)</td>
<td>7.01 (2.28)</td>
<td>7.40 (7.31)</td>
<td>5.15 (32.5)</td>
<td>0.89</td>
</tr>
<tr>
<td>N-16870.213</td>
<td>4.61 (4.88)</td>
<td>7.26 (1.62)</td>
<td>17.2 (5.77)</td>
<td>3.98 (16.1)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

\(E_{\text{max}}\) is maximal drug effect, \(E_0\) is the effect in the absence of drug, \(EC_{50}\) is the value of the PK/PD index required to achieve 50% of \(E_{\text{max}}\), \(\gamma\) is the Hill coefficient and \(R^2\) is the coefficient of determination.

### Table 3. Target values of colistin \(\text{fAUC/MIC}\) for stasis and 1 and 2 log10 kill against all three strains of \textit{P. aeruginosa} and all three strains of \textit{A. baumannii} in the thigh and lung infection models

<table>
<thead>
<tr>
<th>Model/species/strain</th>
<th>Target value of colistin (\text{fAUC/MIC})a</th>
<th>1 log10 kill</th>
<th>2 log10 kill</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thigh infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{P. aeruginosa} ATCC 27853</td>
<td>8.7 (7.9–9.4)</td>
<td>10.9 (10.0–11.8)</td>
<td>13.7 (12.5–15.3)</td>
</tr>
<tr>
<td>PAO1</td>
<td>6.0 (5.9–6.1)</td>
<td>6.6 (6.4–6.7)</td>
<td>7.4 (7.1–7.8)</td>
</tr>
<tr>
<td>19056</td>
<td>7.5 (6.6–8.5)</td>
<td>10.0 (8.8–11.5)</td>
<td>13.5 (11.5–16.4)</td>
</tr>
<tr>
<td>\textit{A. baumannii} ATCC 19606</td>
<td>1.4 (1.1–1.8)</td>
<td>3.5 (2.8–4.2)</td>
<td>9.0 (6.6–14.2)</td>
</tr>
<tr>
<td>248-01-C.248</td>
<td>3.9 (3.1–4.6)</td>
<td>6.0 (5.6–6.5)</td>
<td>7.4 (7.0–7.9)</td>
</tr>
<tr>
<td>N-16870.213</td>
<td>9.3 (8.4–10.2)</td>
<td>13.9 (13.1–14.6)</td>
<td>17.6 (16.7–18.4)</td>
</tr>
<tr>
<td><strong>Lung infection</strong></td>
<td></td>
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<tr>
<td>\textit{P. aeruginosa} ATCC 27853</td>
<td>34.1</td>
<td>43.3</td>
<td>51.8</td>
</tr>
<tr>
<td>PAO1</td>
<td>15.2</td>
<td>44.8</td>
<td>b</td>
</tr>
<tr>
<td>19056</td>
<td>38.6</td>
<td>57.9</td>
<td>b</td>
</tr>
<tr>
<td>\textit{A. baumannii} ATCC 19606</td>
<td>c</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>248-01-C.248</td>
<td>11.6</td>
<td>20.8</td>
<td>36.8</td>
</tr>
<tr>
<td>N-16870.213</td>
<td>c</td>
<td>c</td>
<td>c</td>
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</table>

\(\text{fAUC/MIC}\) values calculated from Monte Carlo simulation \((n=10000, R\) statistical computing package) using parameter estimates in Table 2. IQRs are not provided for lung infection model target values because precise estimates of \(E_{\text{max}}, EC_{50}\) and the Hill coefficient could not be obtained (Figure 4).

\(\text{fAUC/MIC}\) values resulting from the highest tolerated dosage regimens of colistin.

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\(\text{fAUC/MIC}\) values resulting from the highest tolerated dosage regimens of colistin.

\(\text{fAUC/MIC}\) values resulting from the highest tolerated dosage regimens of colistin.
(ATCC 19606 and N-16870.213), for which no reductions in bacterial numbers were observed even at the highest doses able to be administered, are colistin heteroresistant.\textsuperscript{16} This phenomenon is the presence of colistin-resistant subpopulations in a strain that is considered susceptible based upon MIC.\textsuperscript{7} It is possible that colistin heteroresistance accounts for the observed difference in response to colistin across the strains in the lung infection model.

The antibacterial effect of colistin on the \textit{P. aeruginosa} and \textit{A. baumannii} strains following intratracheal inoculation by aerosolization of bacterial suspension in the current study was less than that observed with instillation of bacterial suspension into the nares of mice.\textsuperscript{15,16} As noted above, there was good agreement in the results for \textit{P. aeruginosa PAO1} between the current study and an investigation by another group.\textsuperscript{14} It is possible that the intratracheal aerosolization used in the present study led to establishment of infection in the lower regions of the lungs that was more representative of pneumonia. As with the thigh infection model, the \( \text{fAUC/MIC} \) values for various magnitudes of colistin effect in the lung infection model (Table 3) have been determined based upon the plasma unbound fraction of 0.084 determined by ultracentrifugation and rapid equilibrium dialysis in low-sorption cells.

Across all strains of both species colistin was substantially less effective in decreasing bacterial burden in lungs compared with the antibacterial effect observed in thighs. The refractoriness of experimental lung infection in mice\textsuperscript{15} and piglets\textsuperscript{23} to systemically administered colistin has been reported previously. In contrast, such animal infections are substantially more responsive to nebulized colistin.\textsuperscript{23} This is not surprising given that recent preclinical\textsuperscript{23,28–30} and clinical\textsuperscript{31–33} studies have clearly demonstrated that colistin concentrations achieved in lung tissue, epithelial lining fluid or sputum are very much higher after direct administration to the lungs as compared with the corresponding concentrations achieved after systemic administration. In view of these considerations and the results of a recent systematic review and meta-analysis of clinical studies,\textsuperscript{34} the role of nebulized colistin methanesulphonate for the treatment of pneumonia warrants urgent attention in well-designed clinical studies.\textsuperscript{35}

In summary, this study has provided new information on \( \text{fAUC/MIC} \) targets for various magnitudes of bacterial kill in key animal infection models. The study has also provided information on the relative effectiveness of systemically administered colistin on thigh and lung infections. The results, together with recognition that the plasma unbound fraction is \( \approx 6 \)-fold higher in humans than in mice, will inform consideration of appropriate breakpoints and be used translationally to optimize the clinical use of colistin.

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\textbf{Transparency declarations}

None to declare.

\textbf{References}

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