A pharmacokinetic/pharmacodynamic model developed for the effect of colistin on *Pseudomonas aeruginosa* in vitro with evaluation of population pharmacokinetic variability on simulated bacterial killing

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Objectives: An optimized dosing regimen of the prodrug of colistin, colistin methanesulphonate (CMS), against resistant *Pseudomonas aeruginosa* is needed to ensure effective bacterial killing. The objectives of this study were to develop a pharmacokinetic (PK)/pharmacodynamic (PD) model that characterizes the time course of the antibacterial activity of colistin against *P. aeruginosa* in a static in vitro system and to perform simulations of different dosing regimens and dosing algorithms to evaluate the effect of interindividual variability and interoccasion variability in PK on bacterial killing.

Methods: Static in vitro time–kill curve experiments were conducted on two different strains of *P. aeruginosa* (MIC 1 and 1.5 mg/L). Mechanism-based PK/PD models were fitted in NONMEM7 and the final model was combined with a previously developed population PK model of CMS and colistin to perform simulations of variability based on different dosing algorithms.

Results: A model with compartments for growing and resting bacteria, with a function allowing the maximal bacterial killing of colistin to reduce upon increasing colistin exposure, characterized both the fast bactericidal effect and the adaptive resistance. The variability in PK was shown to translate into pronounced interoccasion variability in bacterial killing. A flat fixed loading dose was demonstrated to result in less variability than an algorithm based on weight.

Conclusions: The developed PK/PD model described the growth, death and resistance development of *P. aeruginosa* in response to colistin for two different strains. Based on simulations, a flat fixed loading dose followed by an 8 or 12 hourly maintenance dose with an infusion duration of up to 2 h appeared adequate.

Keywords: adaptive resistance, loading dose, colistin methanesulphonate

Introduction

*Pseudomonas aeruginosa* is known to possess the capability of developing resistance to multiple antibiotics, be it permanent or temporary (adaptive) resistance.1 As the bacteria cause secondary infection in long-term disease, e.g. cystic fibrosis, and in immunocompromised and critically ill patients, it poses a serious threat with high morbidity and mortality. In dealing with this resistance-prone Gram-negative bacterium, colistin (polymyxin E) has seen increasing clinical use in the last few years. Colistin is an old antibiotic that did not go through rigorous drug development evaluation when it was introduced, but in recent years there have been several studies on the pharmacokinetic (PK)2–6 and pharmacodynamic (PD)7–9 properties of this antibiotic to further aid the development of an optimal dosing regimen.3,10

An advantage of studying antibiotics is the fact that much information on bacterial killing and growth can be gained from in vitro time–kill curve experiments. In these experiments, bacteria can be tested against single or combinations of antibiotics, across different static or dynamic concentrations, with repeated measurements over time. PK/PD models characterizing the observed emergence of resistance may be developed from in vitro data incorporating the mechanisms of resistance for better representation of the drug effect over time. A mechanism-based PK/PD model developed earlier11 that considers the co-existence of growing (drug-susceptible) and resting (non-susceptible) bacteria could be further expanded to incorporate resistance. An example is the PK/PD model developed for adaptive resistance of *Escherichia coli* to gentamicin,12 which may also be applicable to the time course of killing of *P. aeruginosa* by colistin. In addition,
for colistin, its concentration- and time-dependent disappearance in test tubes (because of adherence to the pipette and tube walls and/or degradation) is an important consideration for understanding the link between concentration and the effect on bacteria.

To increase knowledge of how to dose patients, it is important to establish population PK models built on clinical concentration–time data that characterize the variability between patients (interindividual variability, IIV) and within patients on different dosing occasions (intereasion variability, IOV). A population PK model and a PK/PD model based on in vitro data can be combined for predictions and simulations of different dosing regimens that generate concentration–time profiles to drive the PK/PD relationship and bacterial killing. A better understanding of the influence of unexplained and explained variability (e.g. covariates such as weight) can be obtained by comparing bacterial killing with and without these variability components. This approach can help in the development of dosing strategies that not only kill susceptible bacteria but also overcome pre-existing resistance and minimize the development of resistance.

A model that characterizes the PK/PD relationship for colistin in the presence of resistance in vitro would therefore be of value in the evaluation of the efficiency of dosing regimens. In patients, the prodrug colistin methanesulphonate (CMS) has been administered as a flat fixed dose, i.e. the same dose is given to all individuals, with administration typically two or three times daily. Recently, a loading dose of CMS was demonstrated to be needed to quickly achieve therapeutic colistin concentrations, since the formation of colistin from CMS is slow. One study has proposed that duals, with administration typically two or three times daily, would therefore be of value in the evaluation of the efficiency of dosing regimens.

Materials and methods

Bacteria and media

In vitro time–kill curve experiments were conducted for 24 h on two strains of \textit{P. aeruginosa}: wild-type ATCC 27853 (www.atcc.org) and a meropenem-resistant type, ARU552, that had been isolated clinically at the Uppsala Academic Hospital (UAH). MICs of meropenem for the meropenem-resistant type, ARU552, that had been isolated clinically at P. aeruginosa

\textit{In vitro} time–kill curve experiments were conducted without any mechanism for dilution of the colistin concentration, i.e. the colistin concentrations were intended to be constant for the whole 24 h duration of the experiments. All experiments were conducted in 10 mL polypropylene tubes with 4 mL of MH Ca\(^{2+}\)/Mg\(^{2+}\) broth. A starting bacterial concentration of \(\approx 10^8\) cfu/mL was added to each tube. Colistin was then added to the tube to achieve a range of different concentrations, both lower and higher than the MIC. The intended colistin concentrations for the ATCC strain were 0.25, 0.5, 1, 2, 4, 8 and 16 mg/L. For \textit{P. aeruginosa} ARU552, the intended concentrations were 0.375, 0.75, 1.5, 3, 6, 12 and 24 mg/L. For each experiment, a growth control experiment was conducted in which no colistin was added. The tubes were incubated in a temperature-regulated room at 37\(^\circ\)C. Samples were taken frequently, diluted serially and cultured on two to four agar plates. Samples were taken pre-dose and 0.5, 1, 2, 3, 4, 6, 8 and 24 h after the start of the experiments. The blood agar plates were similarly incubated at 37\(^\circ\)C and the number of colonies was counted manually after 18–24 h. For each concentration, the experiments were conducted with two or three replicates on different occasions. The limit of detection (LOD) was 10 cfu/mL.

\textbf{Measured colistin concentration}

Actual colistin concentrations were measured at 0, 8 and 24 h with a previously established analytical method utilizing liquid chromatography–tandem mass spectrometry to evaluate potential colistin degradation and binding to the material of the containers used during the experiments. The rate constant of colistin loss (k\(_d\)) was calculated for each intended concentration between the different sampling times using log linear regression. The measured concentration and the rate constant were used to derive a predicted colistin concentration at any time point during the experiment and this predictive concentration drove the PK/PD model.

\textbf{Semi-mechanistic PK/PD model building}

In the first step, a previously developed semi-mechanistic model describing the development of adaptive resistance of \textit{E. coli} in the presence of gentamicin was applied to the current data. Different functions (linear, power, exponential and a sigmoid E\(_\text{max}\) function) for describing the concentration–effect relationships and the development of resistance were investigated (Figure 1).

In this model, the basic compartments were drug-susceptible, growing bacteria (S) and non-susceptible, resting bacteria (R) with first-order rate constants for growth (k\(_\text{growth}\)) and natural death (k\(_\text{death}\)). The equations below describe the relationship in the absence of antibiotics:

\[
\frac{dS}{dt} = k_{\text{growth}} \times S - k_{\text{death}} \times S - k_S \times S
\]

\[
\frac{dR}{dt} = k_S \times S - k_{\text{death}} \times R
\]
The bacteria are either in a growing and drug-susceptible compartment (S) or in a resting and drug-non-susceptible compartment (R). The bacteria multiply with a first-order rate constant in the susceptible compartment (kgrowth) and all bacteria have a first-order natural death rate (kdeath). The total bacterial content in the system (S+R) stimulates the transfer, determined by a rate constant (kSR), from the growing stage into the resting stage. The colistin compartment (Col), with a first-order elimination rate (ke), drove the killing of the bacteria. In model development, ke was set to the disappearance rate constants for colistin determined from the in vitro experiments and in the simulations this compartment was exchanged with the PK compartment for colistin. The PD model included a function with a rate constant for development (kon) of resistance (ReON) from a non-resistant state (ReOFF), which was dependent on colistin concentration, and a rate constant for return to susceptibility (koff).

The presence of colistin initiated resistance development, i.e. initially the whole fraction of a hypothetical amount was in compartment for no adaptive resistance, ReOFF, and upon colistin exposure the amount started to transfer to a compartment for resistance being present, ReON.

The bacterial count data were transformed into natural logarithms and all data were fitted simultaneously. To avoid the potential bias that may be introduced when modelling average data, all plates with a detectable number of bacteria were included in the data analysis. There was therefore generally more than one bacterial count determination per sampling timepoint and experiment included in the data analysis. There was therefore generally more than one bacterial count determination per sampling timepoint and experiment included in the estimation. The residual error

\[ k_{\text{col}} = k_{\text{drug kill}} \times C_{\text{col}} \]

\[ k_{\text{drug kill}} = \frac{E_{\text{max}} \times C_{\text{col}}}{(C_{\text{col}} + C_{\text{EC50}})} \]

\[ k_{\text{max}} = \frac{E_{\text{max}} \times \text{slope}}{\text{slope} + C_{\text{col}}} \]

\[ k_{\text{col}} = \frac{E_{\text{max}} \times C_{\text{col}}}{(C_{\text{col}} + C_{\text{EC50}})} \]

In addition to variants of the model described above, two other structural models for resistance were evaluated: (i) a function whereby EC50 of colistin at the initiation of the experiment [E(C50)] increases with time and colistin concentration, EC50 = EC50(0) \times (1 - e^{-k \times C_{\text{col}}}), and (ii) a compensatory mutation function.

**Data analysis**

The bacterial count data were transformed into natural logarithms and all data were fitted simultaneously. To avoid the potential bias that may be introduced when modelling average data, all plates with a detectable number of bacteria were included in the data analysis. There was therefore generally more than one bacterial count determination per sampling timepoint and experiment included in the estimation. The residual error
was split into two different components: one consistent difference common to all replicates at the same timepoint and experiment (RES) and one replicate-specific difference (RRES) to avoid bias between the replicates due to correlations. For timepoints at which all dilutions had bacterial counts below the LOD, the probability of the observation being below the LOD was estimated using the M3 method.

The mean tendencies in the population, i.e. the typical parameter values, were estimated along with random effects described by the residual errors. It was assumed that the variability between the experiments was insignificant and therefore no inter-experiment variability was estimated. Model performance was assessed by the evaluation of diagnostic plots and the objective function value (OFV). In order to discriminate between nested models, the difference in OFV was used. The more complex model was selected when the reduction in OFV was at least 10.83 \((P < 0.001\) for 1 degree of freedom). The model was also evaluated by performing visual predictive checks (VPCs) with stratification on the type of experiment and on the colistin concentration, i.e. all observations from duplicate experiments and all measurements were plotted and overlaid with the median and 80% prediction intervals (10th and 90th percentiles) obtained by simulating 1000 replicates of the original dataset. Log likelihood profiling was utilized to obtain standard errors of the parameters.

**Simulations for assessment of patient population variability**

The final parameter estimates from the current colistin PK/PD model for the ATCC strain and a previously developed PK model for CMS and colistin in critically ill patients was used to simulate the drug concentration and bacteria–time profiles, including IIV and/or IOV. The PK model consisted of two compartments for CMS and one compartment for the formed colistin. CMS clearance \((CL_{CMS})\) was 13.1 L/h (IIV 42%, IOV 30%), with CMS inter-compartmental clearance \((Q_{CMS})\) at 206 L/h, a volume of distribution for the central compartment \((V_{1CMS})\) at 11.8 L and the volume of distribution in the peripheral compartment \((V_{2CMS})\) at 28.4 L (IOV 59%). For the formed colistin, colistin clearance \((CL_{Col})\) was 8.2 L/h (IIV 76%, IOV 48%), with a volume of distribution \((V_{Col})\) of 218 L (IOV 48%). There was 100% correlation between \(CL_{CMS}\) and \(CL_{Col}\) and between \(CL_{Col}\) and \(V_{Col}\), although the magnitude of variability differed for \(CL_{CMS}\) and \(CL_{Col}\).

CMS loading doses of 6, 9 or 12 million units (MU) [180, 270 and 360 mg colistin base activity (CBA), respectively] followed by maintenance dosing of 4.5 MU CMS (135 mg CBA) every 12 h, administered as 15 min infusions, were evaluated following inclusion of both IIV and IOV, or IIV only or IOV only. The IIV and IOV values were simulated for three different scenarios: (i) all variability terms included, as in the developed PK model; (ii) variability for CMS or colistin only; and (iii) variability for \(CL_{CMS}\) or \(CL_{Col}\) only. For each scenario and dosing schedule, 1000 patients were simulated. Comparisons of the variability (the 90% prediction interval) were made for the measured variables colistin concentration and log bacterial count. The percentage of simulated patients who achieved 3 log bacterial killing at a certain timepoint (e.g. 12 h) was also computed.

**Simulations for comparison of dosing algorithm**

In order to evaluate the performance of different dosing schedules, the PK/PD model for the ATCC strain was combined with PK models without covariates and with covariates (weight on \(V_{1CMS}\) or \(CL_{CR}\) on \(CL_{CMS}\)) to generate new individuals by stochastic simulations. When covariate relationships were included in the simulation and estimations, the relationships were as suggested earlier:

\[
V_{1CMS} = 11.5 \times \left(\frac{\text{body weight}}{60}\right)
\]

\[
CL_{CMS} = CL_{CR} \times 0.0613 + 1.90 \quad \text{(CLT is total clearance)}
\]

One thousand simulations were conducted in each scenario. The unbound colistin concentrations were simulated to predict the time courses of

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**Figure 2.** Typical observed profile from one experiment for static time–kill curves for \(P. aeruginosa\) exposed to colistin. (a) Time–kill curve experiments for wild-type (ATCC 27853) with concentrations ranging between 0.042 and 12 mg/L. (b) Time–kill curve experiments for a meropenem-resistant clinical isolate (ARU552) with concentrations ranging between 0.081 and 24 mg/L. Control experiments without addition of colistin were also included.
Figure 3. VPCs for the final model with observed bacterial counts (open circles), as well as the median (black continuous line) and the 80% prediction interval (black broken lines) of simulated data. The plots include all growth controls and experiments in a static in vitro system for (a) P. aeruginosa (ATCC 27853) (top two rows) and (b) meropenem-resistant P. aeruginosa (ARU552) (bottom two rows). The indicated concentrations are the initial concentrations as measured by liquid chromatography–tandem mass spectrometry. Grey broken lines represent the starting bacterial inoculum of $4.5 \times 10^5$ cfu/mL (top line), the 3 log kill (middle line) and 10 cfu/mL (bottom line).

Table 1. Population parameter estimates for the final model; the 95% CIs were obtained from log-likelihood profiling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Explanation</th>
<th>P. aeruginosa ATCC 27853 estimate (95% CI)</th>
<th>P. aeruginosa ARU552 estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{growth}$ (h$^{-1}$)</td>
<td>rate constant of bacterial growth</td>
<td>0.919 (0.800–1.05)</td>
<td>0.813 (0.733–0.893)</td>
</tr>
<tr>
<td>$k_{death}$ (h$^{-1}$)</td>
<td>rate constant of natural bacterial death</td>
<td>0.179 (fixed)</td>
<td>—</td>
</tr>
<tr>
<td>$E_{max}$ (h$^{-1}$)</td>
<td>maximal achievable kill rate constant by colistin</td>
<td>282 (129–298)</td>
<td>—</td>
</tr>
<tr>
<td>EC$_{50}$ (mg/L)</td>
<td>colistin concentration that results in 50% of $E_{max}$</td>
<td>1.16 (0.735–1.85)</td>
<td>—</td>
</tr>
<tr>
<td>Slope (L/mgh)</td>
<td>linear function for drug effect</td>
<td>—</td>
<td>15.9 (1.59–25.6)</td>
</tr>
<tr>
<td>$B_{max}$ (cfu/mL)</td>
<td>bacterial count in the stationary phase</td>
<td>2.19 $\times 10^8$ (1.07 $\times 10^8$–3.97 $\times 10^8$)</td>
<td>—</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Hill factor for drug inhibition due to resistance</td>
<td>0.0208 (0.00461–0.363)</td>
<td>—</td>
</tr>
<tr>
<td>$k_{onmax}$ (L/mgh)</td>
<td>maximal achievable resistance rate constant in the presence of colistin</td>
<td>0.824 (0.557–1.27)</td>
<td>4.76 (1.94–14.3)</td>
</tr>
<tr>
<td>C$_{r50}$</td>
<td>antibiotic concentration that results in 50% of $k_{onmax}$</td>
<td>2.61 (1.30–8.18)</td>
<td>105 (25–243)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Hill factor for drug concentration influencing rate constant for the development of adaptive resistance in the presence of colistin ($k_{on}$)</td>
<td>0.791 (0.605–0.982)</td>
<td>—</td>
</tr>
<tr>
<td>$k_{off}$ (h$^{-1}$)</td>
<td>rate constant for bacteria to return to the susceptible state after developing resistance</td>
<td>0.0911 (0.0714–0.112)</td>
<td>—</td>
</tr>
<tr>
<td>RES</td>
<td>residual error (on ln scale)</td>
<td>1.36 (1.24–1.48)</td>
<td>—</td>
</tr>
<tr>
<td>RRES</td>
<td>replicate residual error (on ln scale)</td>
<td>0.475 (0.43–0.496)</td>
<td>—</td>
</tr>
</tbody>
</table>
Variability for both CMS and colistin. 6 MU loading dose then 4.5 MU twice daily.

Variability for both CMS and colistin. 9 MU loading dose then 4.5 MU twice daily.

Variability for both CMS and colistin. 12 MU loading dose then 4.5 MU twice daily.

Variability for CMS only. 9 MU loading dose then 4.5 MU twice daily.

Variability for colistin only. 9 MU loading dose then 4.5 MU twice daily.

Figure 4. Comparison of variability in 1000 simulated patients for unbound colistin concentrations (upper row for each scenario) and for P. aeruginosa count (lower row for each scenario) when including IIV only (left column), IOV only (middle column) and IIV and IOV (right column). Three different loading doses were evaluated [(a) 6 MU CMS (180 mg CBA); (b) 9 MU CMS (270 mg CBA); and (c) 12 MU CMS (360 mg CBA)], followed in all scenarios...
bacterial killing: (i) the impact of different CLCR values in patients (30, 70 and 120 mL/min) for patients with the same weight; (ii) the influence of the time interval between the loading dose and the start time of maintenance dosing (12 versus 24 h); (iii) the influence of infusion duration (15 min, 30 min and 2 h); and (iv) the impact of different patient weights (50, 70, 90 and 120 kg), given they have the same CLCR values. For each scenario, two dosing algorithms were compared: (i) flat fixed CMS dosing (9 MU loading dose then 4.5 MU maintenance dose every 12 h (twice daily) to all patients); and (ii) CMS dosing calculated by previously developed algorithms.6

Loading dose of CBA (mg) = colistin $C_{ss,ave}$target (mg/L) x 2.0 x weight (kg)  
(10)

Daily maintenance dose of CBA (mg) = colistin $C_{ss,ave}$target (mg/L) x\[1.5 \times CLCR (mL/min) + 30\]  
(11)

[$C_{ss,ave}$ target (average steady-state plasma concentration – total concentration) was set at 1.5 mg/L to obtain similar colistin $C_{ss}$ as for a maintenance dose of 4.5 MU twice daily.]

A start inoculum of 4.5 x $10^5$ cfu/mL, i.e. the average value of all starting inocula in the conducted experiments, was assumed in all simulations. The simulated medians and 5th and 95th percentiles for CMS concentration, colistin concentration and bacterial killing were computed at frequent timepoints during 36 h.

Software

Data were analysed using the Laplacian method and ADVAN9 within the population analysis software NONMEM7,24 NONMEM was also used to simulate concentration–time and bacterial count–time profiles. Review of the dataset and graphical evaluation was performed with the Xpose program (version 4)25 and R (version 2.10, www.r-project.org). Simulations and calculations for VPC, and execution of log-likelihood profiling, were performed using Perl speaks NONMEM (PsN) (version 3.5.5).26

Results

Measured colistin concentrations

The measured initial colistin concentrations in the experiments with P. aeruginosa ATCC 27853 were 0.042, 0.19, 0.3, 0.77, 2.2, 4.3 and 12 mg/L. In the P. aeruginosa ARU552 experiments, the initial colistin concentrations were 0.081, 0.25, 0.96, 2.7, 5.1, 11 and 24 mg/L. Except for the measured concentration of 24 mg/L, which was the same as the intended concentration, the measured colistin concentrations were 8.3%–83% lower than intended, with a larger loss the lower the concentration, and there was also a progressive decline in colistin concentration with time, probably due to unspecific binding of colistin to the material of the containers used and possible degradation during the experiments. The application of actual colistin concentrations was important in the characterization of the concentration–effect relationship. As an example, using the measured concentration in the modelling increased the estimated $E_{max}$ for ATCC 27853 by 63% with only minor changes in $EC_{50}$, and the slope value for ARU552 increased by 50%.

Time–kill curve experiments

There were 41 static experiments with a total of 821 data points (where 57 data timepoints entered in the dataset were below the LOD) in the data analysis. Time–kill curves for typical experiments are shown in Figure 2. The bacteria exposed to no colistin or low concentrations of colistin were initially growing exponentially to asymptote, towards a maximum bacterial count of $\sim 10^9$ cfu/mL. Initially, there appeared to be a small reduction in the number of bacteria in all experiments. For ATCC 27853 (Figure 2a), regrowth occurred for concentrations of 0.042–0.77 mg/L while the bacterial count remained below the LOD from 19 h for the concentration of 2.2 mg/L and from 3 h for concentrations of 4.3 and 12 mg/L. Regrowth occurred for all concentrations in the experiments for ARU552, and this strain appeared to be less susceptible than measured by Etest, where the MIC was only 50% higher than for the ATCC strain.

Semi-mechanistic PK/PD model

The model structure of the semi-mechanistic PK/PD model for gentamicin5 could fairly well describe the effect of colistin when all parameters were allowed to be re-estimated. The influence of colistin in killing ATCC 27853 was best described by a basic $E_{max}$ model as there was no improvement in the fit ($\Delta$OFV = 0.9) when $\gamma$ was allowed to be estimated, while a linear model did not result in as good a fit. For ARU552, an $E_{max}$ model was not supported by the data and a linear function ($\gamma$ = 1) was sufficient to describe the concentration–effect relationship. Attempts to apply the same $E_{max}$ parameter for the two strains resulted in a worse fit ($\Delta$OFV increased by 53 U) and therefore different functions were used. The observed emergence of adaptive resistance was best described as a reduced $k_{col}$. The transfer rate constant ($k_{on}$) of non-resistance ($Re_{OFF}$) to resistance ($Re_{ON}$) was described by a sigmoid $E_{max}$ relationship for both strains (Equation 8). A reversal of resistance was estimated based on the current data using a first-order rate constant, $k_{rer}$. As indicated by the VPCs, the model described the observed data well for both strains of P. aeruginosa (Figure 3). The parameter estimates of the final model are listed in Table 1. The growth rate constants ($k_{growth}$) for the two strains were estimated at 0.919 and 0.813 h$^{-1}$ for ATCC 27853 and ARU552, respectively, i.e. the growth rate was 11% lower for the clinical isolate from UAH. For ATCC 27853, colistin had a fast bactericidal effect with an initial maximum killing rate constant ($E_{max}$) of 282 h$^{-1}$ and an $EC_{50}$ of 1.16 mg/L, i.e. at lower concentrations, where the concentration–effect relationship is linear, the slope ($E_{max}/EC_{50}$) would be 243 L/mgh. The linear function for bacterial killing of ARU552 had an estimated slope of 15.9 L/mgh. At an unbound colistin concentration of 1 mg/L, the initial killing rate constant ($k_{col}$) would be 112 L/mgh for ATCC 27853 and 15.9 L/mgh for
Figure 5. Simulated medians of CMS concentration (top panels), unbound colistin concentration (middle panels) and killing of the susceptible strain (ATCC 27853) (bottom panels) for a model not including covariate relationships (left panels) and a model including a covariate (right panels). The dosing algorithms presented by Garonzik et al. were used to determine the doses in the simulations (except for the black continuous line). In scenarios (a), (b) and (c), a flat fixed dose (9 MU CMS load; 4.5 MU CMS twice daily as maintenance) or the calculated doses based on Equations 10 and 11 for a weight-based loading dose (7 MU CMS, body weight 70 kg) and a CLCR maintenance dose (1.9, 3.4 and 5.3 MU CMS, calculated for CLCR values of 30, 70 and 120 mL/min, respectively) were applied. In scenarios (a), (b) and (d) the model included no covariate for the predictions in the
ARU552, indicating a lower susceptibility of the clinical isolate to colistin.

The rate constants of natural bacterial death \( (k_{\text{death}}) \) and bacterial count in the stationary phase \( (B_{\text{max}}) \) were assumed to be the same for both strains. The rate constant for the bacteria to return to the susceptible state after developing resistance \( (k_{\text{off}}) \) was also assumed to have the same value for the two strains as there was no improvement in the fit when allowing them to be different. The rate of development of adaptive resistance was dependent on the colistin concentration as described by a sigmoid \( E_{\text{max}} \) function, with a higher maximal achievable resistance rate constant \( (k_{\text{onmax}}) \) for ARU552 than for ATCC 27853 (4.76 versus 0.824 L/mg/h), although the concentration needed to reach half of \( k_{\text{onmax}} \) was much higher for ARU552 (105 mg/L) than for ATCC 27853 (2.61 mg/L). Colistin concentrations of 1, 12 and 24 mg/L resulted in predicted half-lives for onset of resistance of 5.9, 0.96 and 0.61 h, respectively. The half-life for the bacteria to return to the susceptible state after developing adaptive resistance \( (\ln 2/k_{\text{off}}) \) was estimated to 7.6 h for both strains.

The alternative structural models tested did not fit the data equally well. The model with a compensatory mechanism for development of resistant bacteria had an OFV that was 164 U higher than when the final model was applied. When the resistance was modelled as a time- and concentration-dependent \( EC_{50} \), the OFV was 96 U higher. When a delay or a lag time function was fitted to account for the initial small reduction in the bacterial count there was no significant improvement in the OFV.

**Simulations for assessment of variability**

The simulations showed that the variability arising from IOV was higher than the variability arising from IIV for unbound colistin concentrations and also when propagated to variability in bacterial counts (Figure 4a – c). For a loading dose of 6 MU CMS, 50% of the patients were predicted to achieve 3 log bacterial killing at 24 h, while for a 12 MU loading dose of CMS the majority of patients would have sufficient exposure for 3 log bacterial killing. The IIV and IOV in CMS parameters resulted in low variability in the colistin concentration and bacterial killing profiles (Figure 4d) compared with IIV and IOV in colistin parameters (Figure 4e). The IIV for CMS resulted in lower colistin variability than IOV in CMS, and the same was found for colistin when only variability in colistin parameters was included in the simulations.

**Simulations for comparison of dosing algorithms**

For the model including a \( CL_{CR} – CMS \) relationship (Figure 5a, right panel), log bacterial killing was clearly higher for the \( CL_{CR} \)-based maintenance dosing for patients with reduced renal function \( (CL_{CR} \) of 30 mL/min) compared with those with \( CL_{CR} \) of 120 mL/min (3.0 versus 2.2) at 12 h for a flat fixed loading dose. A 24 h interval between loading and the initiation of maintenance dosing resulted in a tendency to regrowth of bacteria at 18 h for all loading doses tested (Figure 5b). The CMS peak concentrations during maintenance dosing declined with infusion duration and were 14, 12 and 8 mg/L for a 15, 30 and 120 min infusion of 4.5 MU, but all evaluated infusion lengths resulted in similar colistin concentration and bacterial killing profiles (Figure 5c). Body weight as a covariate on V1 for CMS had limited impact on the colistin concentrations, as seen when comparing the predictions for the model with (Figure 5d, right) and without (Figure 5d, left) body weight as a covariate. Importantly, a weight-based loading dose resulted in 2.7 less log bacterial killing at 24 h for a 50 kg compared with a 90 kg patient (Figure 5d).

**Discussion**

In this study a mechanism-based PK/PD model that incorporates resistance development for colistin in two different strains of *P. aeruginosa* was developed. The model showed good ability to characterize the observed time courses of bacterial killing and colistin-driven adaptive resistance for a wide range of concentrations–time profiles, as well as growth to a maximum number of bacteria in the system. \( k_{\text{growth}} \) was found to be different for the two strains, the ATCC strain having a faster rate of growth. A specific value for \( k_{\text{death}} \) could not be identified based on the data in the current study and a fixed value from a prior study was assumed. The model maintained a similar structure for the adaptive resistance determined earlier for gentamicin in *E. coli*, although here the degree of adaptive resistance characterized by \( R_{\text{OE}} \) directly affected the rate constant of drug effect. In addition, an \( E_{\text{max}} \) function for the drug effect was not supported for ARU552, and a linear function was sufficient to describe the data.

Upon introduction of colistin into the in vitro environment there was a fast rate of bacterial killing. At the same time colistin triggered the development of adaptive resistance, which resulted in a graded reduction in the drug effect and thereby a reduced capacity to kill the bacteria. The presented model allows adaptive resistance to be reversible and to return to the original degree of susceptibility when colistin diminishes. The mechanism for temporary or adaptive resistance to colistin has been reported in several studies to involve the modification of lipid A of lipo polysaccharide (LPS) in the outer membrane of the bacterial cell wall. The responsible regulatory systems were indicated to be PhoP-PhoQ and PmrA-PmrB, which modify the net negative charge of LPS and reduce bacterial uptake of the antibiotic, although it has been shown that this mechanism was more relevant for Salmonella than for *Pseudomonas*. Another regulatory system that has been proposed is ParR-ParS, which is required for modification of the operon in the presence of subinhibitory concentrations of polymyxin B and colistin.

The rate of onset of adaptive resistance to colistin was found to be lower compared with the rate characterized earlier for...
gentamicin. This may be because the time required to affect efflux pumps (the mechanism responsible for adaptive resistance to gentamicin) is shorter than that needed to affect LPS. Even though the colistin experiments were set up as a static study in which the antibiotic concentrations were intended to be constant throughout the experiment, colistin concentrations declined with time, as evidenced by the measured concentrations at 0, 8 and 24 h. The decreasing colistin concentrations seen in this study may have helped make it possible to estimate $k_{off}$, despite the lack of dynamic experiments, and estimation of $k_{off}$ also allowed the degree of adaptive resistance to achieve equilibrium. In contrast, in the gentamicin study, nearly complete development of resistance was observed, and recovery from adaptive resistance ($k_{off}$) could not be identified during the study period.

A mechanistic PK/PD model has described the effect of colistin on the growth and killing of P. aeruginosa. The model outlined a more detailed mechanism of action, with binding at target sites on LPS leading to bacterial killing and inhibition of drug effect by signal molecules. This mechanistic model structure required some parameters to be fixed during the estimation. The model included lag compartments, where bacteria do not replicate, and the effect of hypothetical signal molecules. Colistin was hypothesized to trigger the killing of three subpopulations of bacteria (susceptible, intermediate and resistant populations, each of which grows and dies naturally) at different rates, to explain resistance development. The model presented here explains resistance development as a gradual adaptation of the bacteria that would reverse upon drug removal, and was found to characterize the available data well (Figure 3).

In the current model, the regrowth observed was assumed to be due only to adaptive resistance. The current model structure may also be applicable when mutants with non-reversible resistance emerge during an experiment. In such cases the rate constant of the return of the bacteria to the susceptible state after developing resistance ($k_{off}$) would approach zero. In the presence of pre-existing subpopulations of resistant mutants, a model with different growth rates and/or sensitivities to drug exposure for two types of bacteria can explain the regrowth observed at the end of experiments.

Simulations from the PK/PD model developed in this study provided an understanding of the relationship between clinically relevant time courses of antibiotic concentrations and the impact on bacterial killing. In combination with the previously developed PK model and a function for non-linear protein binding of colistin, the model was used to generate unbound concentration–time profiles to drive the concentration–effect relationship. This allowed us to evaluate the effect of different dosing schedules and regimens, which can be useful in developing, adjusting and improving the clinical dosing guidelines. The simulations showed the importance of a loading dose for rapid bacterial killing; at 12 h, 75% of the patients receiving a 12 MU loading dose of CMS achieved 3 log kill compared with 55% and 40% of those receiving 9 and 6 MU, respectively (Figure 4a–c, right panels). Subinhibitory concentrations have been shown experimentally to be associated with bacterial regrowth and increased resistance, which further supports the use of a loading dose.

The simulations utilized a PK model developed previously from data in critically ill patients, which incorporates IIV and IOV for both CMS and colistin, and IOV was shown to impose a larger overall variability than IIV (Figure 4). The large variability in bacterial killing appeared to be caused primarily by IOV in the formation of colistin and/or colistin clearance and the volume of distribution. These simulations suggest that target concentration dosing, where the dose to be administered is calculated using an algorithm based on covariates (e.g. weight, CLCR), may overemphasize the value of individual dose adjustments. In the presence of a large IOV and/or a covariate that changes over time, covariate-based dose adjustment may be less useful to control variability and flat fixed dosing may be a simpler alternative.

A weight-based loading dose was shown to result in much lower bacterial killing in low-weight patients compared with patients weighing >70 kg (Figure 5d). This is not surprising as the central volume of CMS has very limited impact on the time course of the active drug, colistin. A patient’s CLCR value, however, already influences the degree of bacterial killing during the loading dose, with a greater extent of bacterial killing the lower the CLCR, assuming that a CLCR – CLCR relationship exists (Figure 5a and b, right panels). This was also reflected after the maintenance dose, when CLCR-based dosing resulted in more similar profiles when the covariate relationship was assumed to be true and was included in the model for predictions of typical patients (Figure 5a and b). The predictions also showed that an infusion duration of up to 2 h can be recommended based on the current simulations (Figure 5c) as the bacterial killing profiles were similar for a 15 min, 30 min and 2 h infusion. There appeared to be some benefit in increasing the bacterial killing by introducing the maintenance dose before 24 h after the loading dose (compare Figure 5a and b).

The PK/PD model developed here may be applied to other bacterial strains and species, and to other antibiotics, if the mechanisms of resistance are similar. However, parameter estimates are likely to vary, and some experimental data will be needed before applying the current model structure in different settings. It should also be recognized that the time–kill in vitro experiments that generated the data for the development of the PK/PD model were only conducted for a 24 h period and thus model extrapolations much beyond this time period should be made with caution. For example, de novo mutations may appear that are not characterized by the current model structure, and a hollow fibre system would be more suitable for the evaluation of longer treatment durations. In addition, the simulations were performed assuming that the bacteria are exposed to the same concentration–time profile as that predicted in the single distribution compartment of colistin. In a clinical situation the drug distribution may be delayed and the concentration magnitude may differ at the site of the infection, inoculum size will vary and the patient’s immune system may contribute to bacterial killing. Our simulations therefore mimic a system without host defence and with a moderate bacterial load. However, we believe that the simulations add to the understanding of the impact of dosing regimen and variability for bacterial killing.

A clear advantage of applying a PK/PD model based on in vitro data in the development of dosing regimens, compared with the traditional PK/PD indices, is the ability to evaluate changes in the interaction of the drug and the bacteria over time (e.g. from 0 to 24 h). Based on the semi-mechanistic model developed here, the outcome of new conditions, e.g. the evaluation of the time course of bacterial kill following a loading dose or time of initiation of maintenance dosing, was explored. Prediction of the effect of different inocula sizes could easily be predicted as well. The
quantitative characterization of bacterial growth, drug-induced killing and resistance development, and differentiation between them, allows a better understanding of the system. In contrast, the traditional PK/PD indices scrutinize only a single timepoint and thereby provide limited understanding of the full concentration–response relationship.7,17 We have demonstrated earlier that PK/PD indices can be identified based on a semi-mechanistic PK/PD model developed from in vitro data14 and the PK/PD index can therefore be seen as a special case of the PK/PD model predictions. The PK/PD indices also rely on adequate determination of the MIC. Here, the difference between the two strains in the time course of cfu was more pronounced than the small difference in measured MIC, illustrating the crudeness of MIC values for the development of dosing strategies.

A mechanism-based PK/PD model was developed to successfully describe the emergence of adaptive resistance of colistin observed in time–kill curve experiments of two P. aeruginosa strains in vitro. The model was used together with a previously developed PK model to predict colistin concentrations and bacterial killing of different dosing schedules in simulated critically ill patients. Based on the simulations and in view of the large IOV, a flat fixed loading dose followed by an 8 or 12 hourly maintenance dose with an infusion duration of up to 2 h appears adequate for patients with normal or moderately impaired renal function. This study supports the idea that adaptive resistance of colistin plays a role in the efficacy of colistin. The mechanism-based model developed here may therefore be a valuable tool in the development of treatment regimens.

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**Transparency declarations**

None to declare.

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