

Exploring colistin pharmacodynamics against *Klebsiella pneumoniae*: a need to revise current susceptibility breakpoints

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Objectives: Because the pharmacokinetic/pharmacodynamic (PK/PD) characteristics of colistin against Enterobacteriaceae are not well explored, we studied the activity of colistin against *K. pneumoniae* in an *in vitro* PK/PD model simulating different dosing regimens.

Methods: Three clinical isolates of *K. pneumoniae* with MICs of 0.5, 1 and 4 mg/L were tested in an *in vitro* PK/PD model following a dose-fractionation design over a period of 24 h. A high and low inoculum of 10⁷ and 10⁴ cfu/mL with and without a heteroresistant subpopulation, respectively, were used. PK/PD indices associated with colistin activity were explored and Monte Carlo analysis was performed in order to determine the PTA for achieving a bactericidal effect (2 log kill).

Results: The $fAUC/MIC$ ($R^2 = 0.64–0.68$) followed by fC_{max}/MIC ($R^2 = 0.55–0.63$) best described colistin's 24 h log₁₀ cfu/mL reduction for both low and high inocula. Dosing regimens with $fC_{max}/MIC \geq 6$ were always associated with a bactericidal effect ($P = 0.0025$). However, at clinically achievable concentrations, usually below $fC_{max}/MIC = 6$, an $fAUC/MIC \geq 25$ was more predictive of a bactericidal effect. Using a dosing regimen of 9 MU/day, the PTA for this pharmacodynamic target was 100%, 5%–70% and 0%, for isolates with MICs of ≤ 0.5 , 1 and ≥ 2 mg/L, respectively. Dosing regimens that aim for a trough level of 1 mg/L achieve coverage of strains up to 0.5 mg/L (target trough/MIC = 2 mg/L).

Conclusions: Characterization of the pharmacodynamics of colistin against Enterobacteriaceae in an *in vitro* model of infection indicates that a revision of current susceptibility breakpoints is needed. Therapeutic drug monitoring of colistin to achieve pharmacodynamic targets in individual patients is highly recommended.

Introduction

The emergence of MDR Gram-negative bacteria, including carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp), isolates has led to the revival of the use of old antibiotics such as colistin.^{1,2} Understanding the pharmacodynamics of colistin against CP-Kp is important in order to optimize antimicrobial therapy against these infections. Although there are several preclinical pharmacokinetic/pharmacodynamic (PK/PD) studies of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in *in vitro* pharmacokinetic and animal models,³ the PK/PD characteristics of colistin against Enterobacteriaceae have not been extensively explored. Here we focused on the pharmacodynamics of colistin against *K. pneumoniae*, and used an *in vitro* PK/PD model to simulate

different colistin exposures against low and high inocula. We subsequently calculated the PTA for isolates with different MICs to evaluate the susceptibility breakpoint of colistin.

Materials and methods

Isolates, drug and medium

Three clinical isolates of *K. pneumoniae* with colistin CLSI MICs of 0.5 mg/L (WT TZAN59), 1 mg/L (carbapenemase-producing KPC1433) and 4 mg/L (verified in quadruplicate experiments) were used. The isolates were stored at -70°C and revived after subculturing on MacConkey agar plates at 37°C for 18–24 h, and final concentrations of 10⁷ and 10⁴ cfu/mL, verified by quantitative cultures, were used as starting inocula. Colistin sulfate (Sigma Aldrich, Athens, Greece) and cation-adjusted Mueller–Hinton broth

were used according to CLSI instructions.⁴ The two inocula were chosen based on preliminary experiments to assess the presence of heteroresistant subpopulations. Briefly, 100 µL of 10-fold increasing inocula (10^3 – 10^7 cfu/mL) of TZAN59 and KPC1433 isolates were cultured on Mueller–Hinton agar containing 2-fold concentrations of colistin ranging from 2 to 64 mg/L and the number of colonies grown after 24 h were counted and MICs determined. No resistant subpopulations were found at inocula up to 10^4 cfu/mL whereas 1–13 cfu were grown on Mueller–Hinton agar containing 8–32 mg/L with the 10^7 cfu/mL inoculum.

In vitro PK/PD model

A previously developed closed diffusion/dialysis *in vitro* pharmacokinetic model was used in the present study in order to simulate the pharmacokinetics of colistin in humans and to study its antibacterial effect.^{5,6} A dose-fractionation design was followed with nine dosing regimens of colistin targeting fC_{max} 9, 3 and 1.5 mg/L administered every 8, 12 and 24 h for 24 h. High drug exposures were included in order to better describe the exposure–effect relationships with effects ranging from low to high. Colistin was added to both compartments of the *in vitro* model in order to reach a peak concentration within 1.5 h, simulating exposures achieved with a loading dose followed by maintenance doses. The activity of additional dosing regimens with increasing fC_{max} and longer half-lives was also assessed. Drug concentrations in the internal compartment were determined with a microbiological diffusion assay using 10^6 cfu/mL *Escherichia coli* ATCC 25922 impregnated in antibiotic medium 10 agar in Mueller–Hinton broth (Difco, Athens, Greece) (concentration range 0.25–16 mg/L with $r^2 = 0.98$ and inter-day variation <7%).⁷

PK/PD analysis

The PK/PD relationships were analysed by non-linear regression using the E_{max} model described by the equation $E = E_{max} \times (EI/EI_{50})^m / [1 + (EI/EI_{50})^m]$, where E is the bacterial load at the end of experiment (dependent variable) in \log_{10} cfu/mL, EI is the PK/PD index fC_{max}/MIC , $fAUC/MIC$ or $\%fT_{>MIC}$ (independent variable), E_{max} is the maximum bacterial load in \log_{10} cfu/mL observed in the drug-free control group, EI_{50} is the EI corresponding to 50% of E_{max} and m is the slope of the concentration–effect curves (Hill coefficient) (GraphPad Prism 4.03, San Diego, CA). Colistin exposures associated with a bacteriostatic effect (i.e. no change compared with the initial inoculum after 24 h) and a 2 log kill effect (i.e. $2 \log_{10}$ cfu/mL reduction from initial inoculum) were calculated. The 2 log kill effect in the present model was previously found to be associated with 1 log kill in a thigh infection murine model.⁸ In order to capture pharmacodynamic effects for the entire 24 h period, a similar analysis was performed using the area under the 24 h time–kill curve (AUTKC) normalized to span from 100% (drug-free control) to 0% (\log_{10} LOD \times 24). Bactericidal effects were analysed with classification and regression tree (CART) analysis, using as a response the presence of bactericidal activity as the nominal value and all three PK/PD indices as continuous variables. The results of the CART analysis were assessed statistically with Fisher's exact test.

Monte Carlo simulations and analysis

The probability of attaining a 2 log kill effect against *K. pneumoniae* isolates was calculated by applying Monte Carlo simulations of 5000 patients with normal renal function (mean CL_{CR} 85–105) treated with either 9 MU q24h, 4.5 MU q12h or 3 MU q8h. These dosing regimens were previously found to result in $tC_{max} \pm SD$ 5.83 ± 0.87 , 2.98 ± 0.27 and 3.34 ± 0.35 mg/L; total $tC_{min} \pm SD$ 2.60 ± 1.12 , 2.01 ± 0.47 and 1.63 ± 0.23 mg/L; and $tAUC_{0-24} \pm SD$ 72.93 ± 38.57 , 60.71 ± 12.0 and 50.18 ± 10.74 mg·h/L, respectively.⁹ The Monte Carlo simulation was performed using the normal random number generator function of Excel (MS Office 2007) and the corresponding fC_{max} and $fAUC$ were calculated based on the 40% of unbound fraction of colistin in human serum.⁸ The PK/PD PTA associated with a 2 log kill effect was

calculated for each MIC and dosing regimen. The PTA at the epidemiological cut-off (ECOFF) of colistin for *K. pneumoniae* (2 mg/L) was also estimated.

The cumulative fraction of response (CFR)¹⁰ was calculated for *K. pneumoniae* with the WT MIC distribution as presented on the EUCAST web site with the following frequencies: $\leq 1\%$, 20%, 55%, 18% and $\leq 2\%$ for MICs ≤ 0.125 , 0.25, 0.5, 1 and ≥ 2 mg/L, respectively. In addition, the CFR was also calculated for a hypothetical collection of isolates with MICs shifted by one, two and three 2-fold dilutions higher than the EUCAST MIC distribution described above, resulting in three MIC distributions with modal MICs of 1, 2 and 4 mg/L and resistance rates (isolates with MIC >2 mg/L) of 3%, 20% and 73%, respectively. Finally, the trough levels required with each dosing regimen in order to achieve a bactericidal effect for isolates with increasing MICs were calculated taking into account the 40% unbound fraction,⁸ a 1/30 AUC/ C_{min} ratio⁹ and a $t_{1/2}$ of 12 h.^{9,11}

Results

In vitro pharmacodynamics

For the higher inoculum, after a concentration-dependent decrease in \log_{10} cfu/mL within the first 2 h, regrowth was observed for some dosing regimens, in particular for the strain KPC1433 (Figure 1). A 2 log kill effect was observed for the q8h dosing regimen with $fC_{max} \geq 1$ mg/L. For regimens with longer dosing intervals, a bactericidal effect was observed for the q12h dosing regimens with $fC_{max} \geq 4.5$ for KPC1433, and $fC_{max} \geq 0.75$ mg/L for TZAN59 and for the q24h dosing regimens with $fC_{max} \geq 6$ mg/L for KPC1433 and $fC_{max} \geq 3$ mg/L for TZAN59. Resistant subpopulations were found at both $t = 0$ h and $t = 24$ h. The MICs of these populations were higher (32 mg/L) than the MICs of the initial isolates.

For the lower inoculum, a decrease in \log_{10} cfu/mL was also observed within 2 h. However, this was not concentration dependent since all dosing regimens reduced bacterial load to below the LOD (data not shown). No resistant subpopulations were found at $t = 0$ h and $t = 24$ h. The MICs of colonies grown on drug-free media at 24 h were similar to the MICs of initial isolates, whereas the MICs of colonies grown on colistin-containing media were higher.

In vitro PK/PD relationships

The PK/PD relationships for the 24 h \log_{10} cfu/mL and the 24 h AUTKC are shown in Figure 2. For the highest inoculum of 10^7 cfu/mL and the 24 h \log_{10} cfu/mL change, the R^2 for all three PK/PD indices were in the range 0.55–0.68, with the $fAUC/MIC$ showing the highest R^2 . Large variability between the two strains was observed for intermediate exposures ($fAUC/MIC$ 10–30 and fC_{max}/MIC 1–4) with 24 h \log_{10} cfu/mL change varying from 2 \log_{10} growth to 6 \log_{10} kill. This variability was minimized when the 24 h AUTKC was used to express outcome as a function of $fAUC/MIC$, giving a slightly higher R^2 (0.90) compared with fC_{max}/MIC ($R^2 = 0.83$) and $\%fT_{>MIC}$ ($R^2 = 0.79$). The largest variability was again observed at intermediate drug exposures with effects varying from 20% to 80% of E_{max} .

Similar results were found with the lower inoculum with both $fAUC/MIC$ ($R^2 = 0.64$ for 24 h \log_{10} cfu/mL change and $R^2 = 0.76$ for 24 h AUTKC) and fC_{max}/MIC ($R^2 = 0.63$ for 24 h \log_{10} cfu/mL change and $R^2 = 0.62$ for 24 h AUTKC), which clearly offer a better description than $\%fT_{>MIC}$ ($R^2 = 0.51$ for 24 h \log_{10} cfu/mL change and $R^2 = 0.44$ for 24 h AUTKC) (data not shown).

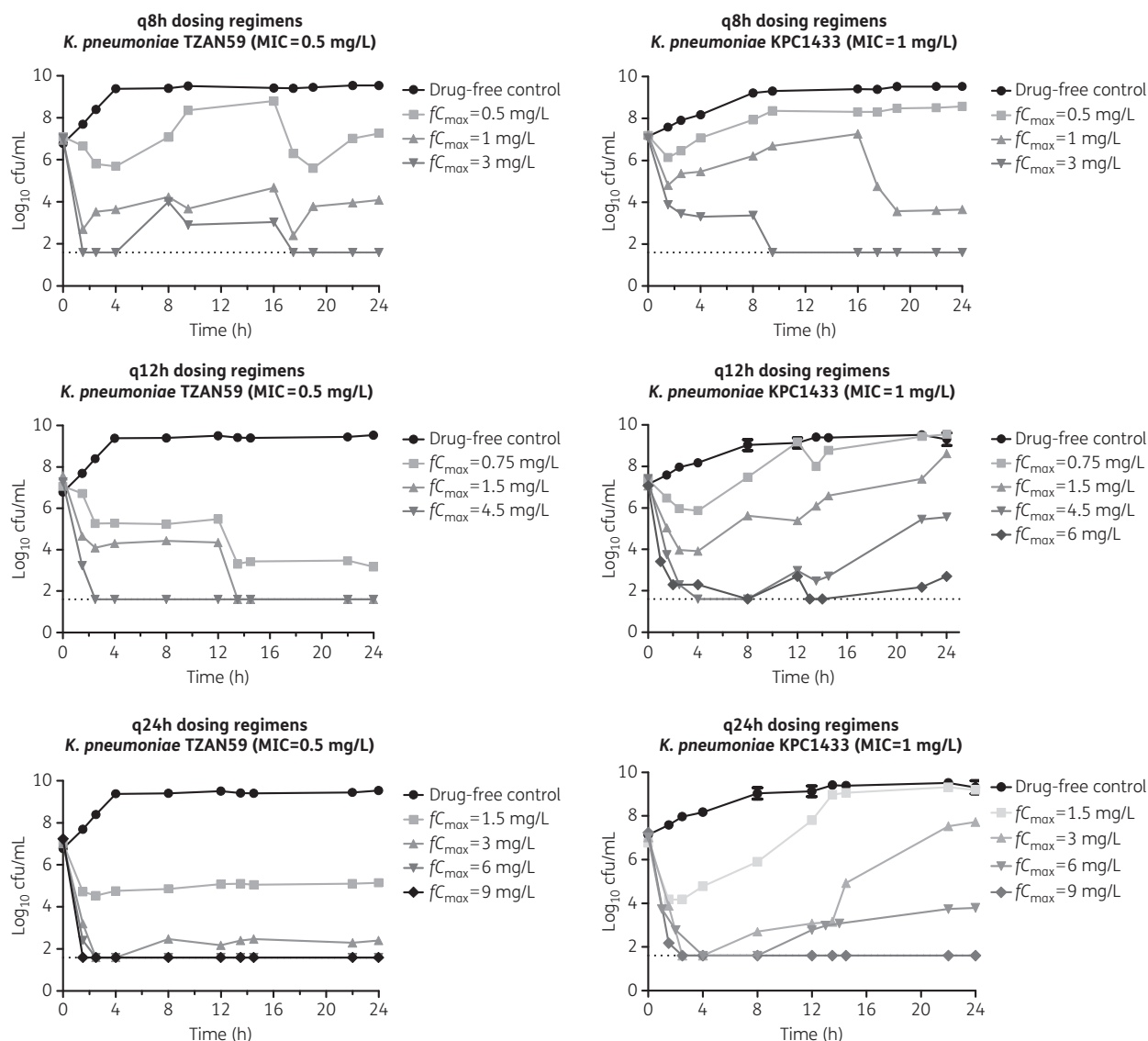


Figure 1. Time-kill curves of initial dose-fractionation studies in the *in vitro* model for the *K. pneumoniae* TZAN59 (left) and KPC1433 (right) isolates using an initial inoculum of 10^7 cfu/mL. The horizontal dotted line represents the lower limit of detection ($1.7 \log_{10}$ cfu/mL). The mean \pm SD difference between measured and target peak concentrations was $13 \pm 5\%$.

Given the relatively small R^2 of exposure-effect relationships, CART analysis was performed in order to determine an EI strongly associated with a 2 log kill effect. CART analysis showed that both fC_{max}/MIC and $fAUC/MIC$ were associated with 2 log kill for the high inoculum. Dosing regimens with an fC_{max}/MIC ratio of ≥ 6 and an $fAUC/MIC$ ratio of ≥ 30 resulted in 100% (10/10) 2 log kill, whereas this was only 38% (6/16) with lower fC_{max}/MIC and $fAUC/MIC$ ratios ($P = 0.0014$). Likewise, for the low inoculum, an $fC_{max}/MIC \geq 6$ ($P = 0.0025$) and an $fAUC/MIC$ ratio of ≥ 25 ($P = 0.013$) was associated with 2 log kill.

In order to elucidate the impact of fC_{max} and $fAUC$ on colistin pharmacodynamics separately, dosing regimens with different fC_{max} and $fAUC$ s were simulated in additional experiments in the *in vitro* model targeting increasing PK/PD index values. A 1 h infusion and a 10 h infusion were applied to achieve fC_{max} 1–6 mg/L and

$fAUC$ s varying from 7.5 to 32 mg-h/L against TZAN59 and KPC1433. Confirming the results in the initial experiments, a high fC_{max}/MIC ratio appeared to have a beneficial effect. A bactericidal effect was found for dosing regimens with $fC_{max}/MIC \geq 6$ against TZAN59 even with an $fAUC/MIC$ as low as 15 (Figure 3). By contrast, for dosing regimens with low fC_{max}/MIC ratios between 1 and 6, bactericidal effects were less apparent and observed only at high $fAUC/MIC$ ratios between 30.6 and 61 (Figure 4a and b). In another set of experiments simulating dosing regimens targeting fC_{max}/MIC ($fAUC/MIC$) ratios of 3 (32), 6 (21), 6 (62) and 12 (42), complete kill was found for all dosing regimens with either $fC_{max}/MIC \geq 6$ or $fAUC/MIC \geq 32$ (Figure 4c and d). The simulated dosing regimens with fC_{max} 32 mg/L q24h and $t_{1/2} = 2$ h (obtained fC_{max}/MIC 6.1 and $fAUC/MIC$ 23.5) and fC_{max} 16 mg/L q12h and $t_{1/2} = 12$ h (obtained fC_{max}/MIC 3.8 and $fAUC/MIC$ 40.6) were

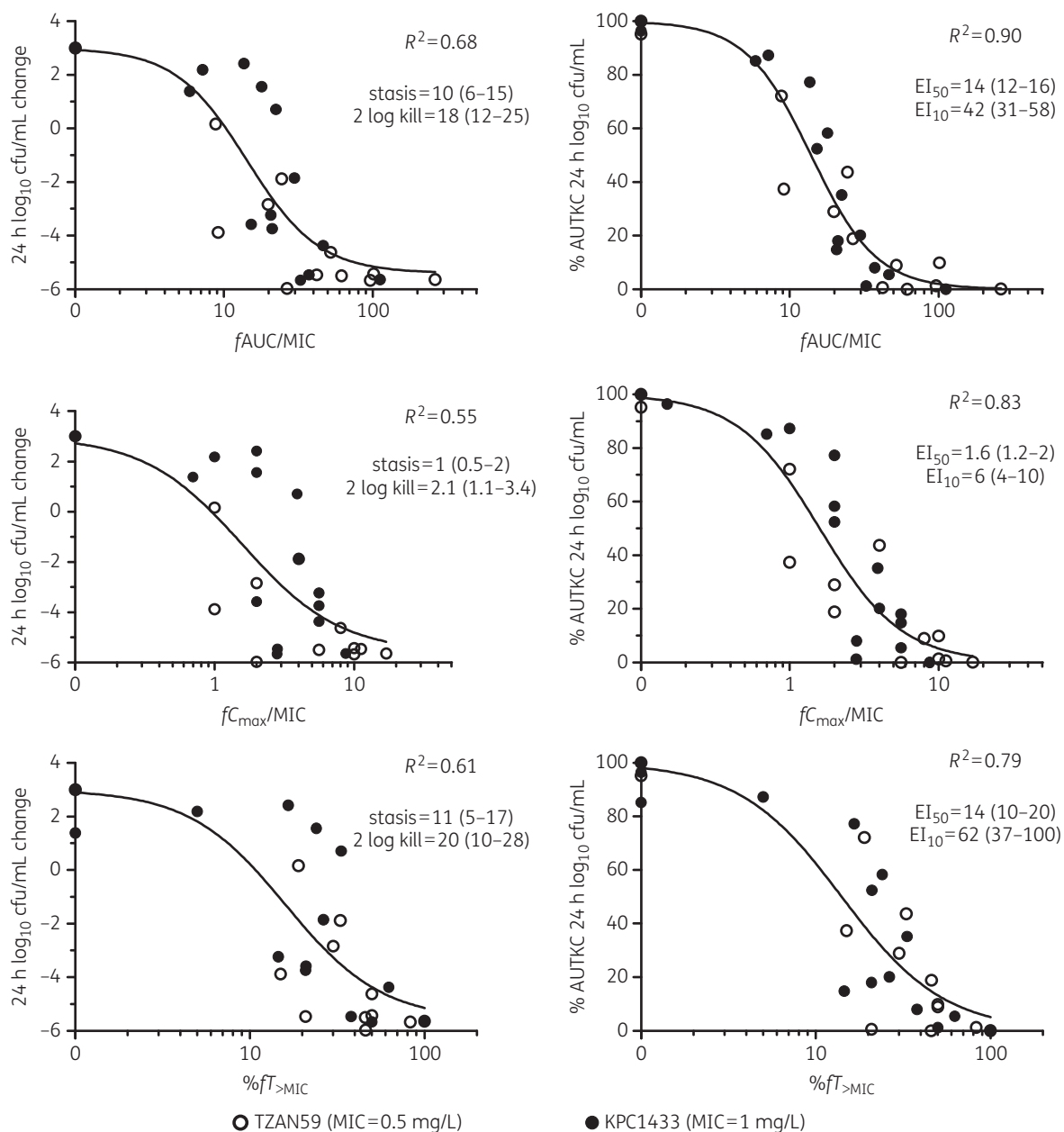


Figure 2. The *in vitro* PK/PD relationships $fAUC/MIC$ (top), fC_{max}/MIC (middle) and $\%fT_{>MIC}$ (bottom) of colistin using the 24 h log₁₀ cfu/mL change compared with the initial inoculum (left graphs) and the normalized percentage area under the 24 h time-kill curve (AUTKC) compared with the AUTKC of drug-free controls (right graphs) using an initial inoculum of 10⁷ cfu/mL.

effective in achieving a 2 log kill effect of the low-level resistant *K. pneumoniae* isolate. Thus, all experiments indicate that both fC_{max}/MIC and $fAUC/MIC$ ratios are important targets for efficacy.

Target attainment

Using the results above, the pharmacodynamic targets $fC_{max}/MIC = 6$ and $fAUC/MIC = 25$ were chosen in order to estimate the PTA since both targets were associated with 2 log kill activity against *K. pneumoniae*. The PTAs are shown in Figure 5 for the three most often used clinical dosing regimens of 9 MU q24h,

4.5 MU q12h and 3 MU q8h. An $fC_{max}/MIC > 6$ was attained for isolates with MIC ≤ 0.25 mg/L for most (>90%) patients treated with 9 MU q24h and for isolates with an MIC ≤ 0.125 mg/L for most patients treated with 4.5 MU q12h and 3 MU q8h (similar results were found with the 3 log kill). However, $fAUC/MIC > 25$ was attained in 100%, 5%–70% and 0% for isolates with MICs of ≤ 0.5, 1 and ≥ 2 mg/L, respectively, with all three dosing regimens (Figure 5). Thus, the PTA was 0 for the current breakpoint of 2 mg/L. The CFRs for *K. pneumoniae* isolates following the EUCAST MIC distribution with a modal MIC of 0.5 mg/L and a resistance rate (isolates with MIC > 2 mg/L) of 2% were comparable for the three

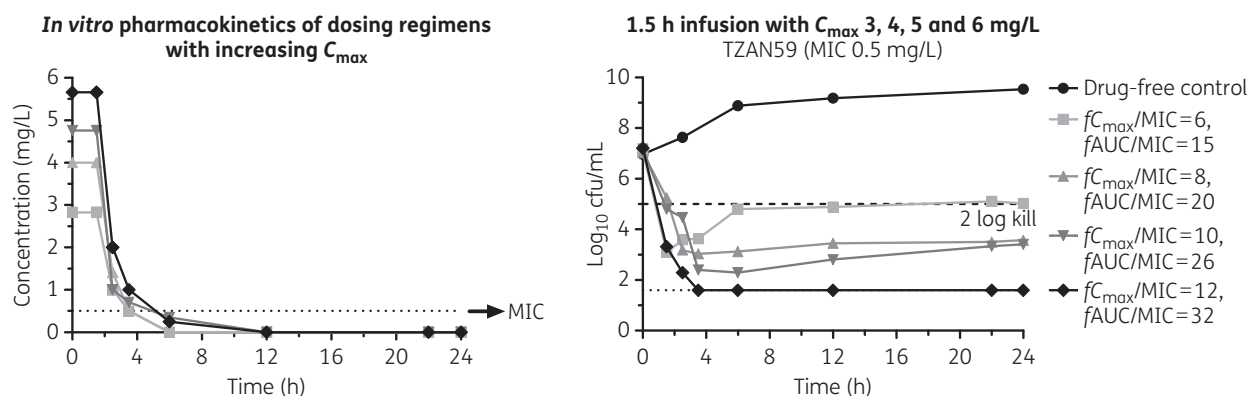


Figure 3. In vitro pharmacodynamics of different dosing regimens with increasing fC_{max} against the WT (TZAN59) *K. pneumoniae* isolate. Left graph: concentration–time profiles of dosing regimens used. Right graph: killing curves of four dosing regimens.

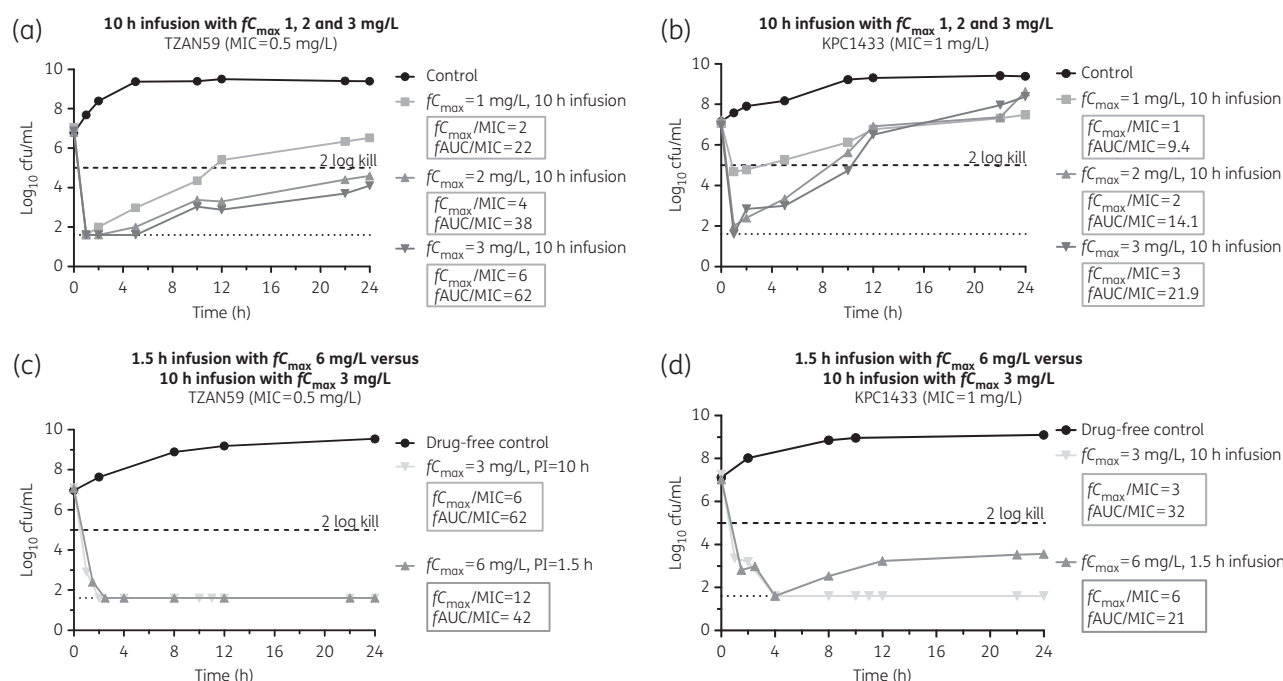


Figure 4. In vitro pharmacodynamics of different dosing regimens with 1.5 and 10 h infusion and increasing fC_{max} against (a, c) the carbapenemase-producing KPC1433 *K. pneumoniae* isolate, and (b, d) a WT (TZAN59) *K. pneumoniae* isolate.

dosing regimens (76%–83%) with the highest CFR found for the 3 MU q8h (Figure 6a). For MIC distributions with higher modal MICs and resistance rates of 1 mg/L and 3% (Figure 6b), 2 mg/L and 20% (Figure 6c), and 4 mg/L and 73% (Figure 6d), the CFR was 82%, 39%, 8% and 0% for 3 MU q8h, respectively.

The trough levels required to attain a 2 log kill effect for isolates with increasing MICs are shown in Figure 7. In order to attain the PK/PD target $fAUC/MIC > 25$, the estimated target trough/MIC ratio was 2, indicating that isolates with an MIC up to 1 mg/L could be covered with a non-toxic dosing regimen (trough levels < 3.33 mg/L).¹² This could just be achieved with 3 MU q8h and 4.5 MU q24h. However, 1 mg/L is below the breakpoint of 2 mg/L and colistin therapy is therefore marginal at best. Most patients treated with 9 MU q24h could achieve those levels but the large interindividual variation and the fact that one-quarter of the

patients would have toxic trough levels indicates the necessity for therapeutic drug monitoring (TDM).

Discussion

In vitro PK/PD modelling of colistin showed that both fC_{max}/MIC and $fAUC/MIC$ described colistin's activity against low and high inocula of *K. pneumoniae*. At low inoculum, regrowth was associated with adaptive resistance since recovered isolates grown in drug-free media had low MICs. In contrast, for the high inoculum, regrowth was associated with heteroresistance since recovered subpopulations of isolates had high MICs. Dosing regimens with $fC_{max}/MIC > 6$ were associated with a > 2 log kill effect independently of $fAUC$. However, for dosing regimens with lower fC_{max}/MIC ratios, an $fAUC/MIC > 25$ appeared to be more predictive of > 2 log

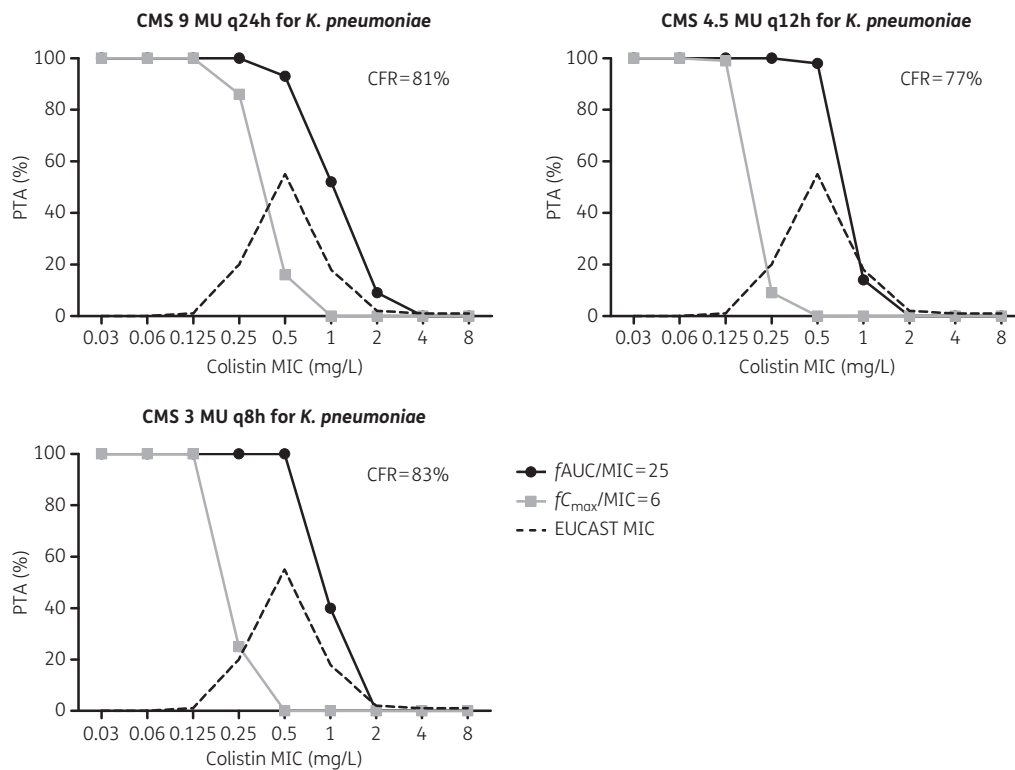


Figure 5. Probability of target attainment for *K. pneumoniae* isolates with increasing MICs and three clinical dosing regimens of colistin administered in ICU patients with normal renal function¹¹ for two PD targets: $fAUC/MIC = 25$ (black line) and $fC_{max}/MIC = 6$ (grey line). The cumulative fraction of response (CFR) is shown for a collection of isolates with the EUCAST MIC distribution with a modal MIC of 0.5 mg/L (broken line).

kill activity. Because conventional clinical dosing regimens usually result in low serum fC_{max}/MIC ratios, the apparently determining PK/PD index in patients is the $fAUC/MIC$. Although the PTA for an $fAUC/MIC >25$ was 100% for isolates with an MIC ≤ 0.5 mg/L, it decreased to 0% at 2 mg/L. The latter is the ECOFF for *K. pneumoniae*, indicating that colistin is likely not optimally effective as monotherapy. The clinical breakpoint at present, however, is 2 mg/L.¹³ The CFR for WT isolates was 82%, and dropped significantly when shifts in distributions were simulated.

The PK/PD properties of colistin against Enterobacteriaceae have not been previously explored in detail. Preclinical studies on using colistin in animals against *P. aeruginosa* and *A. baumannii* showed that $fAUC/MIC$ best correlated with bacterial killing. The $fAUC/MIC$ required for a 1 log₁₀ kill in animal thigh and lung infections models was 12.2–22.8 for *P. aeruginosa* and 7–42 for *A. baumannii*.³ This is in agreement with the $fAUC/MIC$ found in the present study against *K. pneumoniae*. In a previous animal pneumonia model using *K. pneumoniae*, a colistin AUC/MIC of 158.5 was associated with 68% mortality.¹⁴ Since colistin protein binding in mouse serum is 90%–92%,¹⁵ the $fAUC/MIC$ should be 12–16, which is within the $fAUC/MIC$ range found in the present study.

Although $fAUC/MIC$ accurately describes colistin activity, fC_{max}/MIC was also closely correlated with colistin activity, particularly for the low inoculum, in which no heteroresistant subpopulations were found. For the higher inoculum, including heteroresistant subpopulations, the $fAUC/MIC$ was strongly correlated with colistin activity. However, a concentration-dependent killing was observed within 2 h with an $fC_{max}/MIC >6$ possibly required to

kill the more susceptible subpopulation without affecting the less susceptible subpopulation for which a high $fAUC/MIC >25$ was required in order to prevent regrowth. This may explain why CART analysis indicated both fC_{max}/MIC and $fAUC/MIC$ as equally significant predictive PK/PD indices for killing the high inoculum. Colistin's concentration-dependent killing was previously described against *P. aeruginosa* and *A. baumannii*^{16,17} and an $fC_{max}/MIC >8$ –10 was suggested for other concentration-dependent bactericidal drugs,¹⁸ although $fAUC/MIC$ appears to be the pharmacodynamic driver.^{15,19} Similarly, single high doses of azithromycin, an $fAUC/MIC$ -dependent drug, were more efficacious than multidose regimens in preclinical infection models.²⁰ In contrast to other concentration-dependent drugs, colistin exerts a strong initial concentration-dependent killing but no significant post-antibiotic effect at low, clinically relevant concentrations (1 h at the MIC and 2–3 h at $>16 \times MIC$).^{16,21} In addition, colistin has a relatively long half-life. However, given that a high fC_{max}/MIC cannot be obtained in patients' serum for isolates with an MIC >0.25 mg/L, the $fAUC/MIC$ may be the determining PK/PD index in patients.

Based on a PK/PD target of 25 for the $fAUC/MIC$, Monte Carlo simulations showed a cumulative fraction of response for the WT distribution of 77%–83% for all three clinical dosing regimens. These rates are similar to the clinical cure rate of 82.1% found in patients with sepsis due to Gram-negative bacteria susceptible only to colistin and treated with 4.5 MU q12h.²² A similar cure rate of 83.3% was reported in another retrospective cohort study.²³ However, lower clinical cure rates (57%–73%) have been reported in several retrospective studies in which lower colistin

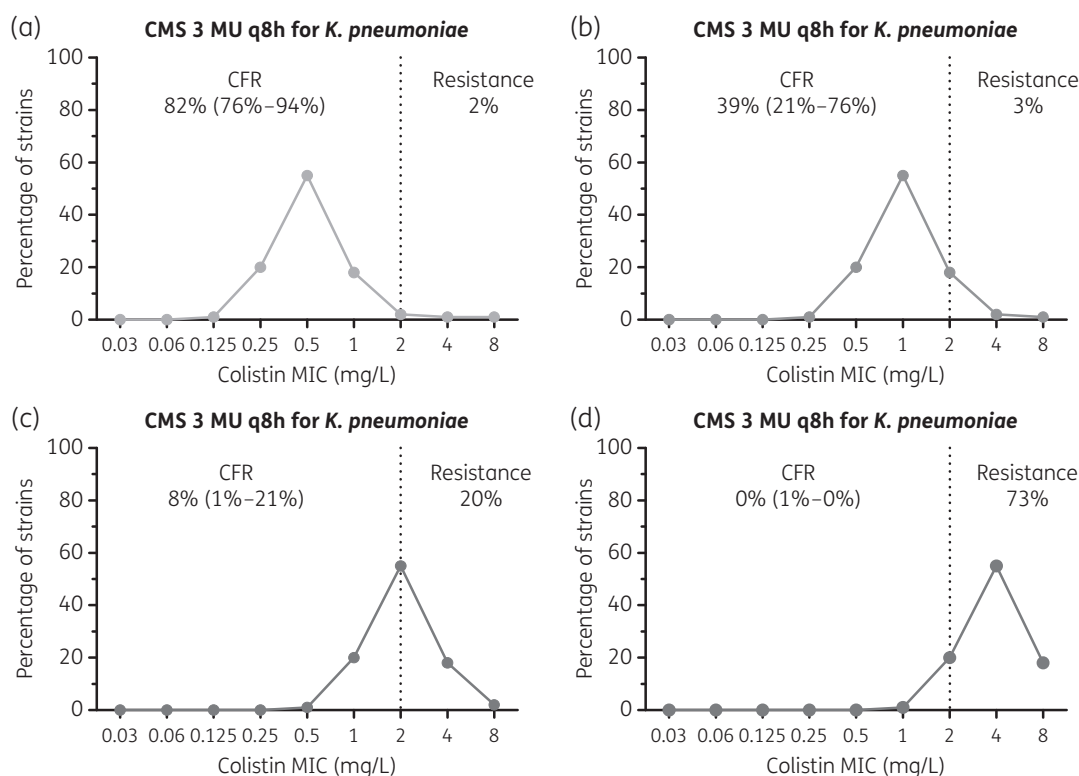


Figure 6. The cumulative fraction of response (CFR) defined as the population PTA for the 3 MU q8h CMS dose (similar exposures were found for 4.5 MU q12h and 9 MU q24h) in ICU patients with normal renal function¹¹ and a collection of isolates with modal MICs of 0.5 (EUCAST MIC distribution), 1, 2 and 4 mg/L, with proportions of resistant isolates (MIC > 2 mg/L, horizontal dotted line) of 2%, 3%, 20% and 73%, respectively.

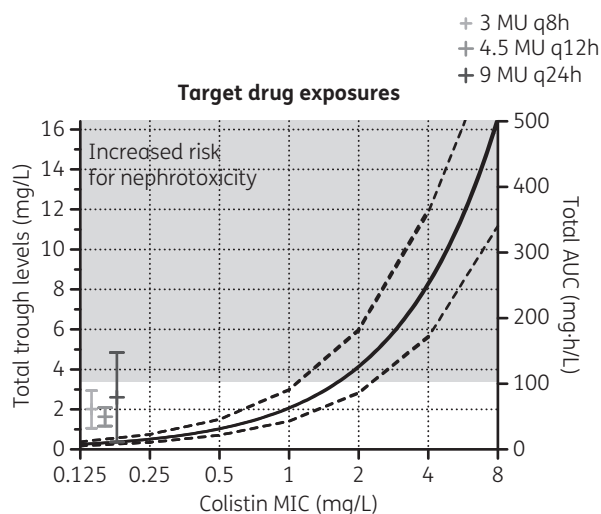


Figure 7. Target drug exposure (total trough levels on the left axis and total AUC₀₋₂₄ on the right axis) required to attain the mean (95% CI) PK/PD target $fAUC/MIC$ of 25 (17–36) and the target $tAUC$ of 62.5 (42.5–90) against *K. pneumoniae* isolates with different MICs. The shaded area indicates increased risk for nephrotoxicity, which is usually associated with trough levels > 3.33 mg/L. The dotted lines represent the 95% CI of the PK/PD target calculated from the *in vitro* model. Trough levels of the three clinical dosing regimens 9 MU q24h, 4.5 MU q12h and 3 MU q8h in ICU patients with normal renal function⁹ are shown next to the left-hand axis.

methanesulphonate (CMS) doses (1–3 MU) were given to patients with ventilator-associated pneumonia.¹ Such low daily doses would not produce drug exposures sufficient to attain the PK/PD target for isolates with an MIC of 0.5–1 mg/L.

The lower success rates reported with colistin may be explained by inefficient drug exposure or infections by less susceptible isolates shifting out of the WT distribution. A recent surveillance multicentre study recording resistance rates for colistin of *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *A. baumannii* isolates from ICU patients showed that although the resistance rate is low (<10%), the median MIC is 1 mg/L,^{24,25} one 2-fold dilution higher than the median MIC of the WT EUCAST distribution. As shown in the present study, this increase reduced the PTA rates significantly. In centres with such a shift in MICs or with higher resistance rates,²⁶ clinical cure by colistin monotherapy is low and therefore alternative chemotherapeutic approaches are followed, e.g. combination therapy.²⁷ Comparable high clinical cure rates were found for colistin monotherapy and combination therapy for isolates with an MIC of ≤ 0.5 mg/L.²⁸ Significantly, this MIC was found in the present study to be the highest MIC offering a reasonable PTA.

The optimal dose should be sufficient to result in a tC_{max} 7.5 mg/L ($fC_{max} = 3$ mg/L) or $tAUC$ of 31.25 mg-h/L ($fAUC = 12.5$ mg-h/L) for an isolate with an MIC of 0.5 mg/L (target $fC_{max}/MIC = 6$ and $fAUC/MIC = 25$). Although high tC_{max} s up to 23 mg/L have been reported,²⁹ few patients could attain such a high tC_{max} of 7.5 mg/L even with the highest dose of 9 MU.⁹ Thus, the $tAUC$ of 31.25 mg-h/L is a more clinically feasible target for isolates with an MIC of up to

0.5 mg/L. Assuming a half-life of 12 h, a trough/MIC ratio of 2 should be targeted in order to obtain a $tAUC$ of 31.25 mg·h/L. Isolates with an MIC of up to 1 mg/L, which represent 94% of the WT distribution, could be treated with a dosing regimen that produces a trough level of 2 mg/L. Isolates with higher MICs would require higher trough levels (>3.33 mg/L), which are associated with an increased risk of nephrotoxicity.¹² An average steady-state trough level of >2 mg/L has been previously suggested as adequate for treatment of these infections.³⁰ However, the large interindividual variability in $C_{ss,avg}$ (median 2.35, range 0.24–9.92 mg/L) and in protein binding (mean \pm SD unbound fraction 0.49 ± 0.11 in critically ill patients)³¹ indicates that most patients would attain the PK/PD target for isolates with an MIC of up to 0.5 mg/L, whereas for isolates with an MIC of 1 mg/L, TDM can optimize drug exposure by increasing efficacy and reducing toxicity. Alternatively, high infrequent doses (e.g. 12–18 MU q48h or q72h) may optimize both C_{max}/MIC and AUC/MIC indices and reduce trough levels associated with nephrotoxicity, although toxicity studies are required to prove the safety of those dosing regimens.

Based on the PK/PD target of $fAUC/MIC >25$, a susceptibility PK/PD breakpoint of ≤ 0.5 mg/L was determined in the present study. This breakpoint is lower than the EUCAST clinical breakpoint and ECOFF value of ≤ 2 mg/L. However, using the latter endpoint, $>98\%$ of isolates would be deemed susceptible, whereas the success rate of colistin monotherapy hardly reaches 80% against colistin-susceptible isolates based on previous clinical studies.²² Of note, using the 0.5 mg/L susceptibility breakpoint, 78% would be susceptible. Furthermore, using the 2 mg/L breakpoint, no difference was found in mortality between carbapenem-resistant Enterobacteriaceae infections by colistin-susceptible and colistin-resistant isolates.³² More important is the finding that the clinical outcomes of colistin therapy in patients infected with susceptible (MIC ≤ 2 mg/L) *A. baumannii* isolates but high colistin MICs (1–2 mg/L) were poorer than in patients infected with isolates with lower (<1 mg/L) MICs (7 day outcome 38% versus 20.2%, $P = 0.025$).³³ Finally, it was suggested that current maintenance doses may not be effective against isolates with an MIC >0.5 mg/L.³⁴ Thus, a revision of the current susceptibility breakpoint for colistin may be needed.

For isolates with an MIC ≤ 0.5 mg/L ($<50\%$ of the clinical isolates) monotherapy can attain the pharmacodynamic target, whereas for isolates with an MIC of 1 mg/L, TDM can optimize drug exposure in order to attain the pharmacodynamic target. Target attainment rates may be higher for patients with reduced renal clearance though with increased risk for toxicity. For those patients, dose adjustments have been recommended based on creatinine clearance.³³ Monotherapy will not be sufficient for isolates with higher MICs and combination therapy should be considered in order to decrease the pharmacodynamic target by synergistic interactions. For patients with augmented renal function, higher CMS doses (12–18 MU) may be used in order to achieve adequate levels of colistin. Those dose recommendations together with the nomogram of Figure 7 can be used in order to optimize CMS dosing regimens for patients with altered renal clearance. However, dose adjustments based on MICs require reliable and reproducible MIC testing assays and the *in vitro* PD target determined here needs to be comparable with the clinical PD target.

In conclusion, *in vitro* PK/PD modelling of colistin activity against *K. pneumoniae* showed that $fAUC/MIC$ is the best predictor of

colistin activity for clinically achievable concentrations, and fC_{max}/MIC the best determinant of colistin bactericidal activity. Colistin rapidly kills susceptible subpopulations in a concentration-dependent manner at $fC_{max}/MIC >6$, whereas for prolonged suppression of growth of resistant subpopulations an $fAUC/MIC >25$ is required. An $fC_{max}/MIC >6$ can be attained with standard dosing regimens for isolates with an MIC ≤ 0.125 mg/L, but very few of these exist. Because of the low fC_{max} achieved in human plasma, $fAUC/MIC >25$ is a more clinically feasible target. Target attainment rates drop rapidly for isolates with reduced susceptibility to colistin, necessitating TDM and the use of combination therapy.

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Transparency declarations

None to declare.

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