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Evaluating the optimal time for amikacin administration with respect to haemodialysis using an in vitro pharmacodynamic simulation against epidemic nosocomial OXA-48 producing *Klebsiella pneumoniae* ST405 strains

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ABSTRACT

Objectives: Bacterial viability and enrichment of resistance resulting from three different amikacin administration schedules with respect to haemodialysis (HD) were assessed against three OXA-48-producing *Klebsiella pneumoniae* isolated during an outbreak in a Spanish hospital.
Methods: A previously described two-compartment dynamic system was used. Three possible amikacin administration schedules were simulated: (i) haemodialysis immediately after amikacin infusion (pre-HD); (ii) infusion immediately after haemodialysis (post-HD); and (iii) infusion at 50% interdialytic period. Amikacin standard dose (SD) and double dose (DD) were simulated for each schedule. Values of C_{max}/MIC, C_{max}/MPC (mutant prevention concentration), $AUC_{0–48h}$/MIC, $AUC_{0–48h}$/MPC and $\%T_{MSW}$ (percentage of time that the concentration was inside the mutant selection window) were determined with experimental data and were correlated with the area under the bacterial killing curve of the total population and the resistant subpopulation.
Results: Both with SD and DD, the pre-HD schedule resulted in increases at 48 h in bacterial counts of the total population at the expense of enrichment of pre-existing resistant subpopulations from 12 h onwards for all strains. The estimated $\%T_{MSW}$ that prevented enrichment of resistant mutants was <61.5%. The $AUC_{0–48h}$/MPC (with values of ≈40 being associated with countering of increases in resistant subpopulations) was better than the $\%T_{MSW}$ as a predictive parameter.
Conclusion: This study showed that the longest times concentrations were above the MPC (i.e. highest $AUC_{0–48h}$/MPC, lowest $\%T_{MSW}$), the lowest enrichment of resistant subpopulations. This implies use of the highest possible amikacin dose (limited by toxicity) and post-HD as the best administration schedule.

1. Introduction

Between April 2010 and December 2011, a hospital-wide outbreak caused by OXA-48-producing *Klebsiella pneumoniae* (OXA48KP) occurred in Hospital Universitario La Paz (Madrid, Spain) [1]. The most frequent sequence type (ST) was ST405, followed by ST11 and ST15 [1]. OXA48KP belonging to ST405 carried different resistance genes [2], clustered in two different

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plasmids [3], favouring intra-/inter-patient diffusion and clonal dissemination [4]. *Klebsiella pneumoniae* ST405 is increasingly present in Spain [5], together with ST15 [6] and ST11 [7], the major clone in Madrid [8].

Among the 62 infected patients (32.8% presenting septic shock/severe sepsis), mortality was 43.5%, being 69.5% among patients with bloodstream infection [1]. In an outbreak context, end-stage renal disease and patients undergoing dialysis represent a high-risk condition, which has been identified as an independent risk factor for multidrug-resistant (MDR) Gram-negative nosocomial infection [9], having a high impact on morbidity and mortality [10]. In the occurring outbreak, amikacin was part of the antibiotic regimens used, with dosing schedules targeted to achieve, against the epidemic OXA48KP strains, previously described optimal pharmacokinetic/pharmacodynamic (PK/PD) values [11–14]. For patients undergoing dialysis, amikacin was administered every 2 days after haemodialysis, as classically [15,16], without PK/PD determinations. No studies have investigated the possibility of increasing antibacterial effects in patients undergoing dialysis by changing the antibiotic administration time with respect to haemodialysis. In the current situation of potential hospital outbreaks involving MDR enterobacteria in the short-term future, investigations aimed at obtaining specific data for patients undergoing dialysis could represent a practical way to prepare for critical scenarios.

The aim of this study was to assess, in an in vitro PK/PD simulation, bacterial viability and enrichment of resistance resulting from different amikacin administration schedules in relation to conventional haemodialysis in patients with end-stage renal failure, against three OXA48KP isolates from the outbreak.

2. Materials and methods

2.1. Strains

Three OXA48KP strains isolated from three patients undergoing haemodialysis owing to severe chronic renal insufficiency were used. Isolates were chosen based on their sequence type: two isolates (strains 1 and 2) belonged to the main sequence type causing the outbreak (ST405); and one isolate (strain 3) belonged to ST11. Strains 1 and 2 carried $bla_{\text{TEM-1}}$, $bla_{\text{SHV-76}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-48}}$, *qnrB1, aac(6′)-Ib*-cr and *aacC3* genes [1–3]. Strain 3 carried $bla_{\text{SHV-11}}$, bla_{ACT}, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-48}}$, *qnrB1*, *aac(6′)-Ib*-cr, *aac(3′)-IIa, aadA2* and *aph(3′)-Ia* [8]. Resistance to aminopenicillins, piperacillin/tazobactam, second- and third-generation cephalosporins, ertapenem, gentamicin, tobramycin and quinolones following European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints was reported for the three strains. All three strains were susceptible to amikacin [minimum inhibitory concentration (MIC) = 4 μg/mL], tigecycline and colistin, and strains 1 and 2 were resistant to fosfomycin, imipenem and meropenem.

Before the study, amikacin MICs and minimum bactericidal concentrations (MBCs) were determined five times in broth [17,18], also using the high inocula used in the simulations. Mutant prevention concentrations (MPCs) were determined [19]. Briefly, strains were cultured overnight in cation-adjusted Mueller–Hinton broth (CA-MHB) (Becton Dickinson & Co., Franklin Lakes, NJ, USA) and the suspensions obtained were centrifuged (4000 × *g*, 10 min) and were re-suspended to obtain bacterial loads of $\geq 5 \times 10^{10}$ CFU/mL. Plates with known amikacin concentrations (8–128 μg/mL) were inoculated and were incubated at 37 °C for 48 h for colony counting (limit of detection, 10 CFU/mL). The MPCs were estimated by linear regression using at least the last three points of the $\log_{10}$ CFU/mL of viable bacteria–amikacin concentration curves. The MPC was defined as the point where the plot intercepted the lower limit of detection (x for $y = 1$ $\log_{10}$ CFU/mL).

2.2. Kinetic simulations

Three schedules of amikacin administration were simulated: (i) amikacin administration followed by haemodialysis immediately after infusion (pre-HD); (ii) amikacin administration immediately after haemodialysis (post-HD); and (iii) amikacin administration at the 50% interval time between two haemodialysis sessions (50% interdialytic period) (mid-HD).

Two doses of amikacin were simulated for the three administration schedules: (i) the standard dose (SD) achieving a peak concentration (C_{max}) of ≥30 μg/mL (i.e. 33.6 μg/mL at the end of the infusion) and a trough concentration (C_{min}) of <5 μg/mL (4.6 μg/mL); and (ii) a double dose (DD) achieving a target C_{max} of ≥60 μg/mL (67.2 μg/mL at the end of the infusion) and C_{min} of <10 μg/mL (9.3 μg/mL). Target values of area under the concentration–time curve from 0 to 48 h ($AUC_{0–48h}$) were 370.7 μg h/mL (pre-HD), 846.7 μg h/mL (mid-HD) and 1154.0 μg h/mL (post-HD) for the SD, and 741.3 μg h/mL (pre-HD), 1693.4 μg h/mL (mid-HD) and 2308.0 μg h/mL (post-HD) for the DD.

PK parameters of amikacin and other aminoglycosides in patients under standard haemodialysis are quite scarce and old [20,21]. In addition, more recent studies in different populations show a wide range of PK parameters [22–31]. Therefore, we selected those parameters [volume of distribution (V_d), clearance, half-life ($t_{1/2}$)] within the range described in the literature and providing the usually considered target C_{max} and C_{min} [30]. So, a monocompartmental model was assumed with a V_d equal to 0.25 L/kg and a constant of elimination (K_e) equal to 0.01386 h^{-1} ($t_{1/2}$ = 50 h) during the interdialytic period and equal to 0.3465 h^{-1} ($t_{1/2}$ = 2 h) during haemodialysis. DD target concentrations were calculated using the same kinetic parameters as for SD. The considered duration of haemodialysis was 4 h.

2.3. In vitro kinetic system (Fig. 1)

A previously described two-compartment dynamic system exposing bacteria to changing study drug concentrations and avoiding dilution of the bacterial inoculum together with the drug was used [31,32]. The central compartment consisted of a spinner flask, the lumina of the capillaries within the dialyser (FX50 class; Fresenius Medical Care S.A., Madrid, Spain) and the tubing in between. The infection site was represented by the extracapillary space of the dialyser unit combined with the intradialyser circulating tubing. The high surface-area-to-volume ratio of the dialysis unit (>200 cm^2/mL) yielded rapid equilibration of the amikacin concentration between the two compartments. Before each experiment, the central compartment was filled with CA-MHB. Amikacin was injected into the central compartment using a computer-controlled syringe pump over a 0.5-h infusion. Decay of amikacin concentration in the central compartment was achieved by a continuous dilution-elimination process using computerised peristaltic pumps (MasterFlex™; Thermo Fisher Scientific, Waltham, MA, USA) set to a rate of 2.6 mL/min and 0.1 mL/min to simulate the elimination half-lives in human serum during dialytic and interdialytic periods, respectively. Flow rates were controlled and exchanged in peristaltic pumps using the MasterFlex Linkable Instrument Control Software (WinLin v.2; Thermo Fisher Scientific). Control drug-free simulations were performed using a fixed rate in peristaltic pumps of 0.1 mL/min. Additional pumps circulated the antimicrobial medium mixture at 50 mL/min between the central and peripheral compartments and at 20 mL/min within the extracapillary space through the external tubing. Both compartments were maintained at 37 °C during the simulation process.

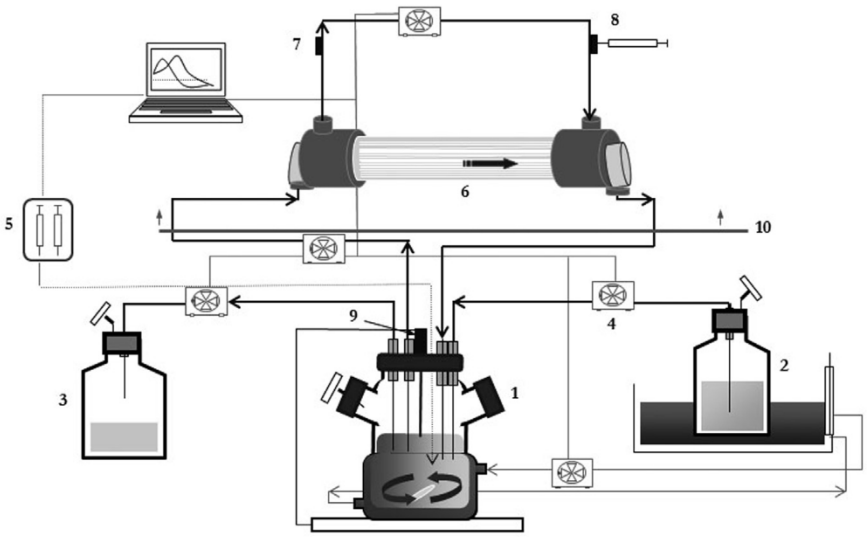

Fig. 1. Diagram of the in vitro computerised device.

2.4. Pharmacokinetic analysis

Aliquots (0.5 mL) were taken from the peripheral compartment at 0, 2, 4, 6, 8, 10, 24, 26, 28, 30, 32, 34 and 48 h and were stored at −80 °C until use. Concentrations were determined by bioassay [33] using Antibiotic medium 1 (Becton Dickinson & Co.) and *K. pneumoniae* ATCC 13883 as indicator organism. Standards and dilutions were prepared in the same broth employed in the PK simulation and were added to wells in the plates with an even lawn of the indicator organism. Plates were incubated at 37 °C for 24 h. The assay was linear ($r^2 > 0.99$) over the range tested (2–75 μg/mL): limit of detection, ≤0.5 μg/mL; and intraday and interday coefficient of variation, <3.64%. The PK parameters $t_{1/2}$, C_{max}, C_{min} and $AUC_{0–48h}$ (log-linear trapezoidal rule) were determined by a non-compartmental approach for constant infusions using Phoenix WinNonlin v.6.3 (Certara, Princeton, NJ, USA).

2.5. Measurement of antimicrobial activity and enrichment of resistant subpopulations

Colonies from an overnight culture were grown in CA-MHB to approximately 2.5×10^8 CFU/mL as measured by an ultraviolet–visible (UV-Vis) spectrophotometer (GBC Scientific Equipment, Braeside, VIC, Australia). Then, 7 mL was inoculated into the peripheral compartment of the system 30 min prior to the start of PD experiments. Final initial inocula were $3–5 \times 10^7$ CFU/mL ($3–5 \times 10^9$ CFU/system). Samples (0.5 mL) for bacterial counting were collected from the device at 0, 2, 4, 6, 8, 10, 24, 26, 28, 30, 32, 34 and 48 h, were diluted and were spread using a spiral plater workstation (Wasp II; Don Whitley, Shipley, UK) onto antibiotic-free Mueller–Hinton agar (Becton Dickinson & Co.) and onto agar containing 3 × MIC amikacin concentrations to quantify amikacin-resistant subpopulations. Plates were incubated for 24 h at 37 °C for colony counting using an automatic bacterial counter (EC2™; bioMérieux, Marcy-l'Étoile, France) (limit of detection, 1.7 $\log_{10}$ CFU/mL). Each experiment was performed in triplicate.

Log_{10} reductions in initial inocula ($\log_{10}$ CFU/mL at time 0 – $\log_{10}$ CFU/mL at each sampling time) were calculated. The area under the bacterial killing curve from 0–48 h ($\log_{10}$ CFU/mL × h^{-1}) was also determined as a measure of global killing along the experimental time [32,34] for the total population (AUBKC) and for the resistant subpopulations ($AUBKC_m$) as a measure of enrichment of bacterial resistance [35]. AUBKC and $AUBKC_m$ were determined by the trapezoidal rule for values above the lower limit of detection using GraphPad Prism 7.0 software (GraphPad Software Inc., La Jolla, CA, USA).

2.6. Pharmacokinetic/pharmacodynamic indices and AUBKC correlation analysis

Values of C_{max}/MIC, C_{max}/MPC, $AUC_{0–48h}$/MIC, $AUC_{0–48h}$/MPC and $\%T_{MSW}$ [percentage of time that concentration was inside the mutant selection window (MSW)] were determined with experimental data.

An inhibitory sigmoid dose–response model (Eq. (1)) was used to fit AUBKC data versus $AUC_{0–48h}$/MIC:

$$y = y_{max} - (y_{max} - y_0)[x^b/(x^b + x_{50}{}^b)] \quad (1)$$

where y is the AUBKC; y_0 and y_{max} are the minimum and maximum values of y, respectively; x is the $AUC_{0–48h}$/MIC; x_{50} is the $AUC_{0–48h}$/MIC corresponding to 50% of maximal effect ($y_{max} - y_0$); and b is the shape parameter.

The relationship between $AUBKC_m$ versus $AUC_{0–48h}$/MIC and $AUC_{0–48h}$/MPC ratios was fitted using a Gaussian-type function (Eq. (2)):

$$y = a \exp\{-0.5[(x - x_m)/b]^2\} \quad (2)$$

where y is the $AUBKC_m$; x is the $AUC_{0–48h}$/MIC or $AUC_{0–48h}$/MPC; x_m is the $AUC_{0–48h}$/MIC or $AUC_{0–48h}$/MPC value at the centre of the distribution; a is the y_{max}, which corresponds to the height of the centre of the distribution; and b is the parameter of the width of the distribution, in the same units as x.

To relate the enrichment of resistant mutants with the $\%T_{MSW}$, the $AUBKC_m$–$\%T_{MSW}$ data that meet the condition $T_{>MPC} > 0$ were fitted using Eq. (1).

The $AUC_{0–48h}$/MIC, $AUC_{0–48h}$/MPC and $\%T_{MSW}$ values preventing enrichment of resistant mutants were estimated for each strain separately and for the combined data from all three strains by interpolating the minimal quantifiable $AUBKC_m$ value (minimal number of quantifiable bacteria, in $\log_{10}$ CFU/mL, above the limit of

detection over a 48-h period), 4 log [(CFU/mL) × h] from the Gaussian or sigmoid curves.

The extra-sum-of squares F test (GraphPad Prism) was used to compare the goodness-of-fit of dose–response curves fitted by the model for the separate and combined data sets (Eq. (3)):

$$F = [(SS_c - SS_s)(DF_c - DF_s)]/(SS_s/DF_c) \quad (3)$$

where SS is the sum-of-squares, DF is the degrees of freedom, SS_c and DF_c are the SS and DF obtained by fitting all data into a single data set, and SS_s and DF_s are the SS and DF obtained by fitting data separately. The *P*-value was calculated from the F ratio, DF_c–DF_s and DF_c. A *P*-value of <0.05 indicated bacterial strain-specific differences in the fitted curves and therefore significantly better fits for separate data.

Table 1
In vitro susceptibility data (μg/mL) of the three study strains.

Strain	ST	Standard inoculum (5×10^5 CFU/mL)		High inoculum ($3–5 \times 10^7$ CFU/mL)		MPC (10^{10} CFU/mL)
		MIC	MBC	MIC	MBC	
1	ST405	4	4	8	8	29.5
2	ST405	4	8	8	8	38.9
3	ST11	4	4	8	8	29.9

ST, sequence type; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MPC, mutant prevention concentration.

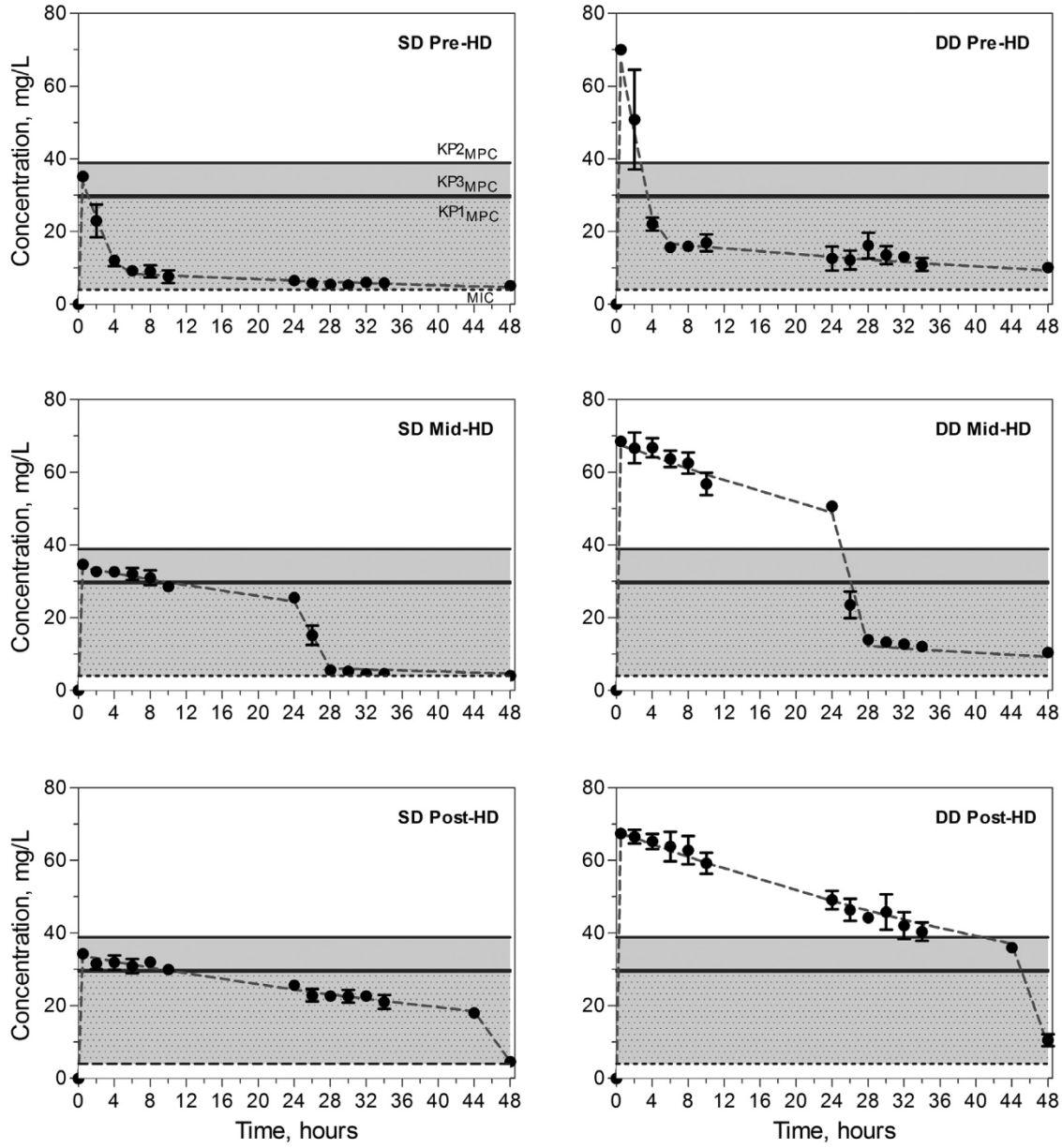

Fig. 2. Experimental pharmacokinetic profiles for the two doses with the three amikacin administration schedules. Values of MIC (– – –) and MPC (––––) are indicated by horizontal lines delimitating the mutant selection windows (MSW). MIC, minimum inhibitory concentration; MPC, mutant prevention concentration; SD, standard dose, DD, double dose; HD, haemodialysis.

3. Results

Table 1 shows the results of in vitro susceptibility tests. Strain 1 (ST405) and strain 3 (ST11) presented the same MICs and MBCs with both inocula as well as MPCs, whilst strain 2 (ST405) presented higher MBCs and MPCs.

Fig. 2 shows experimental PK profiles for the three administration schedules with the two doses tested and the MSWs. Experimental levels matched target levels. As expected, the different dialytic and interdialytic periods of amikacin elimination produced similar values of C_{max} (34.7 ± 0.4 μg/mL for the SD and 68.7 ± 0.8 μg/mL for the DD) and C_{min} (4.6 ± 0.5 μg/mL for the SD and 10.4 ± 0.1 μg/mL for the DD), but markedly different values of $AUC_{0–48h}$: 374.7 ± 22.7 μg h/mL (pre-HD), 840.0 ± 29.8 μg h/mL (mid-HD) and 1161.8 ± 6.4 μg h/mL (post-HD) for the SD; and 762.0 ± 10.3 μg h/mL (pre-HD), 1723.1 ± 27.4 μg h/mL (mid-HD) and 2295.2 ± 34.8 μg h/mL (post-HD) for the DD.

Values of C_{max}/MIC were ≈8 (SD) and ≈17 (DD) for all strains. The C_{max}/MPC was <1 (SD) for all strains and ≈1 (DD) for strains 1 and 3 (being <1 for strain 2). Table 2 shows the values of $AUC_{0–48h}$/MIC, $AUC_{0–48h}$/MPC and $\%T_{MSW}$ for the three strains with the two doses and the three administration schedules.

3.1. Bacterial growth curves

At the beginning of the simulations, the initial inocula were 3–5 × 10^7 CFU/mL with <1 × 10^3 CFU/mL of resistant subpopulations recovered in plates containing 3 × MIC amikacin, both in antibiotic-free (controls) and antibiotic simulations. In antibiotic-free simulations, total bacterial counts increased ≈1.5–2.5 $\log_{10}$ CFU/mL over 48 h, with increases in resistant subpopulations of ≈0.5–1.0 $\log_{10}$ CFU/mL. Figs. 3–5 show $\log_{10}$ CFU/mL over 48 h obtained using the different amikacin administration schedules with the two doses tested for strains 1, 2 and 3, respectively. For all strains, whether the SD or the DD dose was simulated, the pre-HD administration schedule resulted in increases at 48 h in bacterial counts of the total population at the expense of growth of the resistant subpopulations from approximately 12 h onwards. Bacterial loads similar to the initial inoculum (strains 1 and 2) or 1.0 $\log_{10}$ CFU/mL increases (strain 3) were observed at 48 h with the mid-HD schedule and the SD at the expense of resistant subpopulations. In contrast, this schedule (mid-HD) provided no growth of resistant subpopulations with the DD, without differences with the post-HD schedule with the DD. No significant growth of resistant subpopulations with respect to initial inocula could be observed with the post-HD schedule and the SD for strains 1 and 3, but not for strain 2 where resistant subpopulations increased ≈2 $\log_{10}$ CFU/mL at 48 h.

Table 2
$AUC_{0–48h}$/MIC, $AUC_{0–48h}$/MPC and $\%T_{MSW}$ for the three study strains with the two amikacin doses tested.

	Amikacin dose and administration schedule[a]					
	SD			DD		
	Pre-HD	Mid-HD	Post-HD	Pre-HD	Mid-HD	Post-HD
$AUC_{0–48h}$/MIC	93.7	210.0	290.5	190.5	430.8	573.8
$AUC_{0–48h}$/MPC						
Strain 1	12.7	28.5	39.4	25.8	58.4	77.8
Strain 2	9.6	21.6	29.9	19.6	44.3	59.0
Strain 3	12.5	28.1	38.9	25.5	57.6	76.8
$\%T_{MSW}$						
Strain 1	98.3	81.5	76.3	93.1	47.1	6.6
Strain 2	99.9	99.9	99.9	94.6	48.7	22.4
Strain 3	98.4	82.2	79.0	93.2	47.2	6.7

$AUC_{0–48h}$, area under the concentration–time curve from 0 to 48 h; MIC, minimum inhibitory concentration; MPC, mutant prevention concentration; $\%T_{MSW}$, percentage of time that the concentration was inside the mutant selection window; SD, standard dose; DD, double dose.

[a] Pre-HD, amikacin administration followed by haemodialysis immediately after infusion; post-HD, amikacin administration immediately after haemodialysis; and mid-HD, amikacin administration at the 50% interval time between two haemodialysis sessions (50% interdialytic period).

3.2. Relationships of the antimicrobial effect and of the enrichment of resistance to pharmacokinetic/pharmacodynamic indices

The increasing $AUC_{0–48h}$/MIC ratio (from pre-HD to post-HD and/or by increasing the dose from SD to DD) correlated well (r^2 = 0.93–0.99 individually vs. r^2 = 0.98 for the combined three-strain data; P = 0.94) with decreasing values of AUBKC (Fig. 6). The $AUBKC_m$–concentration ($AUC_{0–48h}$/MIC and $AUC_{0–48h}$/MPC) curves were bell-shaped. For each strain, the $AUBKC_m$ value increased (as increased the enrichment of resistant mutants) reaching maximum values with the pre-HD schedule and the SD, and decreased to zero by increasing the AUC of the simulated amikacin schedules. The Gaussian-like function strongly fitted the $AUBKC_m$–$AUC_{0–48h}$/MIC and $AUBKC_m$–$AUC_{0–48h}$/MPC data for each organism (r^2 = 0.91–0.99 for both) and for the combined strain data ($r^2 \geq 0.90$ and 0.94, respectively) (Fig. 7a,b). However, despite the scarce stratification of AUBKC–concentration data, individual $AUBKC_m$–$AUC_{0–48h}$/MIC fits were significantly better (P = 0.036) than the single fit combining all data sets. Unlike the $AUC_{0–48h}$/MIC, the $AUBKC_m$ relationship with $AUC_{0–48h}$/MPC was strain-independent (P = 0.23 for separate versus combined data). Table 3 shows the values of $AUC_{0–48h}$/MIC and $AUC_{0–48h}$/MPC that, according to the model, countered the enrichment of resistant subpopulations.

The $AUBKC_m$ relationship with $\%T_{MSW}$ was modelled using data from the amikacin administration schedules resulting in amikacin concentrations above the MPC ($T_{>MPC} > 0$). Eq. (1) fitted these data quite well (r^2 = 0.94; Fig. 7c). A weaker correlation was found for the $\%T_{MSW}$–$AUBKC_m$ relationship using the entire data set ($T_{>MPC} = 0$ and $T_{>MPC} > 0$; r^2 = 0.70). The estimated $\%T_{MSW}$ (for $T_{>MPC} > 0$) that prevented the enrichment of resistant mutants was <61.5%. This value was similar for strain 1 ($\%T_{MSW}$ <62.2%; r^2 = 0.98) and strain 3 ($\%T_{MSW} < 62.5\%$; r^2 = 0.94); the analysis could not be done for strain 2 owing to insufficient $T_{>MPC}$ data.

4. Discussion

The present study was designed to investigate in vitro alternative amikacin administration schedules with respect to haemodialysis in order to increase antibacterial activity and the prevention of resistance emergence in the face of new bacterial threats. The results pointed to $AUC_{0–48h}$/MPC as the most predictive parameter and to the post-HD schedule as the optimal administration schedule to counter enrichment of resistant subpopulations.

The strains used in this study represented not only isolates from the outbreak but also sequence types of OXA48KP strains circulating in Spain. The described multidrug resistance traits associated with the infecting clones obliged treating physicians to consider combination antibiotic therapy, including amikacin (as the sole aminoglycoside to which isolates were not resistant, although in the susceptibility limit) together with two other available drugs (colistin and tigecycline) according to the susceptibility profile. The PK targets ($AUC_{0–24h}$ and C_{min}) for aminoglycosides such as amikacin to optimise antibiotic dosing regimens and minimise toxicity have been widely described [11,12,14,36]. However, in patients with end-stage renal failure undergoing conventional haemodialysis, optimisation of a dosing regimen to obtain the desired C_{max} and C_{min} is a difficult task, especially considering that the extracorporeal clearance of the drugs significantly changes pharmacokinetics [11]. Specifically,

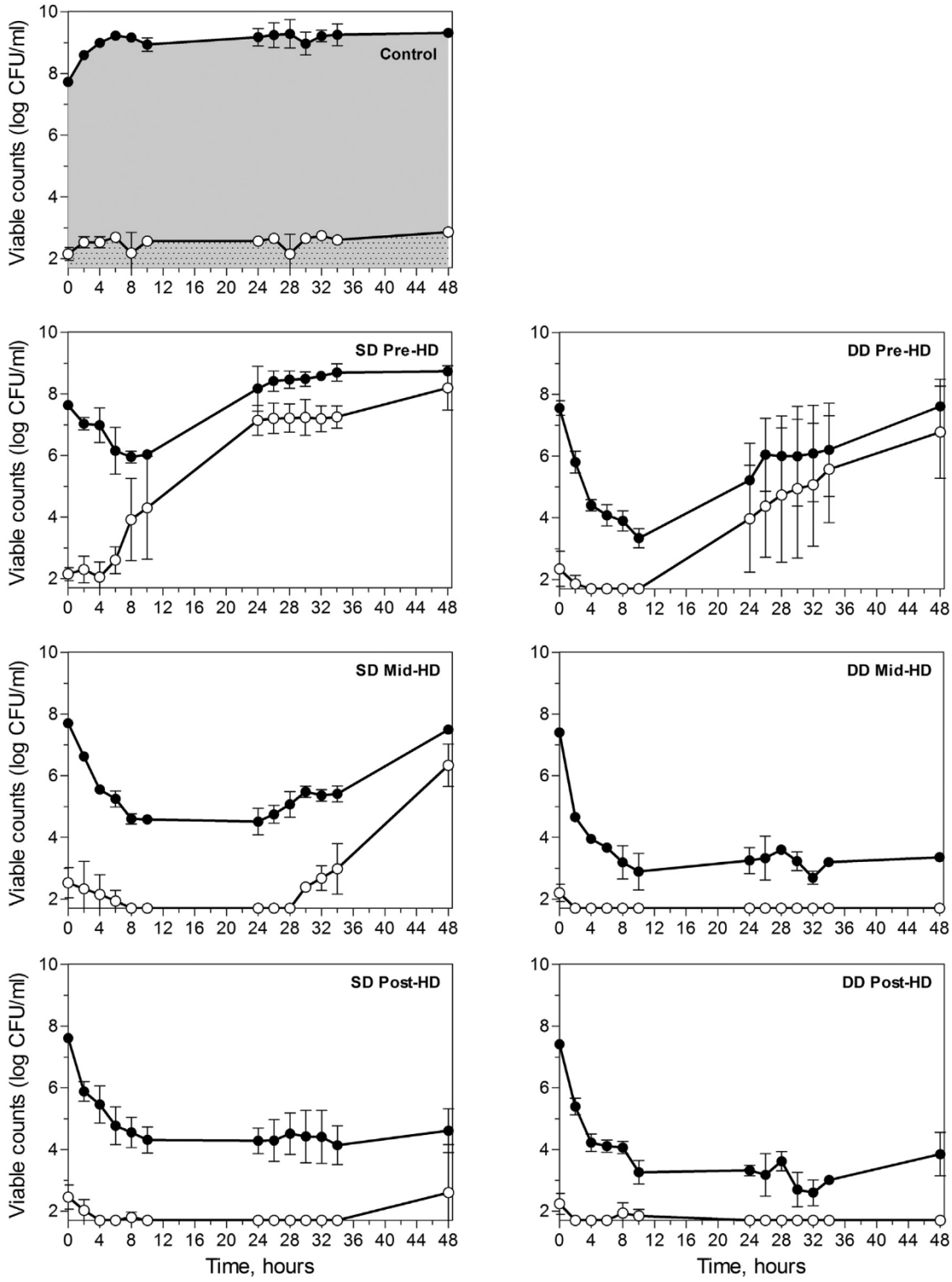

Fig. 3. Bacterial counts (log_{10} CFU/mL) over 48 h obtained using the different amikacin administration schedules with the two doses tested for strain 1 (ST405): bacterial counts over time for the total population (grown in antibiotic-free plates, ●) and the resistant subpopulation (grown in plates containing 3 × MIC of amikacin, ○). As an example, the AUBKC (shaded area) and $AUBKC_m$ (dotted area) are represented in control curves. ST, sequence type; MIC, minimum inhibitory concentration; AUBKC, area under the bacterial killing curve for the total population; $AUBKC_m$, area under the bacterial killing curve for the resistant subpopulations; SD, standard dose, DD, double dose; HD, haemodialysis.

there are no recent studies with amikacin in this type of patient and the dialysis methods used in old studies [21] are not comparable with those used presently. Nowadays, increasing antimicrobial resistance stresses the need for PK/PD data supporting new strategies for the optimisation of amikacin dosing.

Classically, for aminoglycosides it has been established that a C_{max}/MIC ratio of 8–10 or an AUC_{0-24h}/MIC ratio of ≥70–90 are needed for an optimal dosing regimen [11]. These estimations successfully predict the microbiological results for treatments directed to the predominant susceptible population but may be

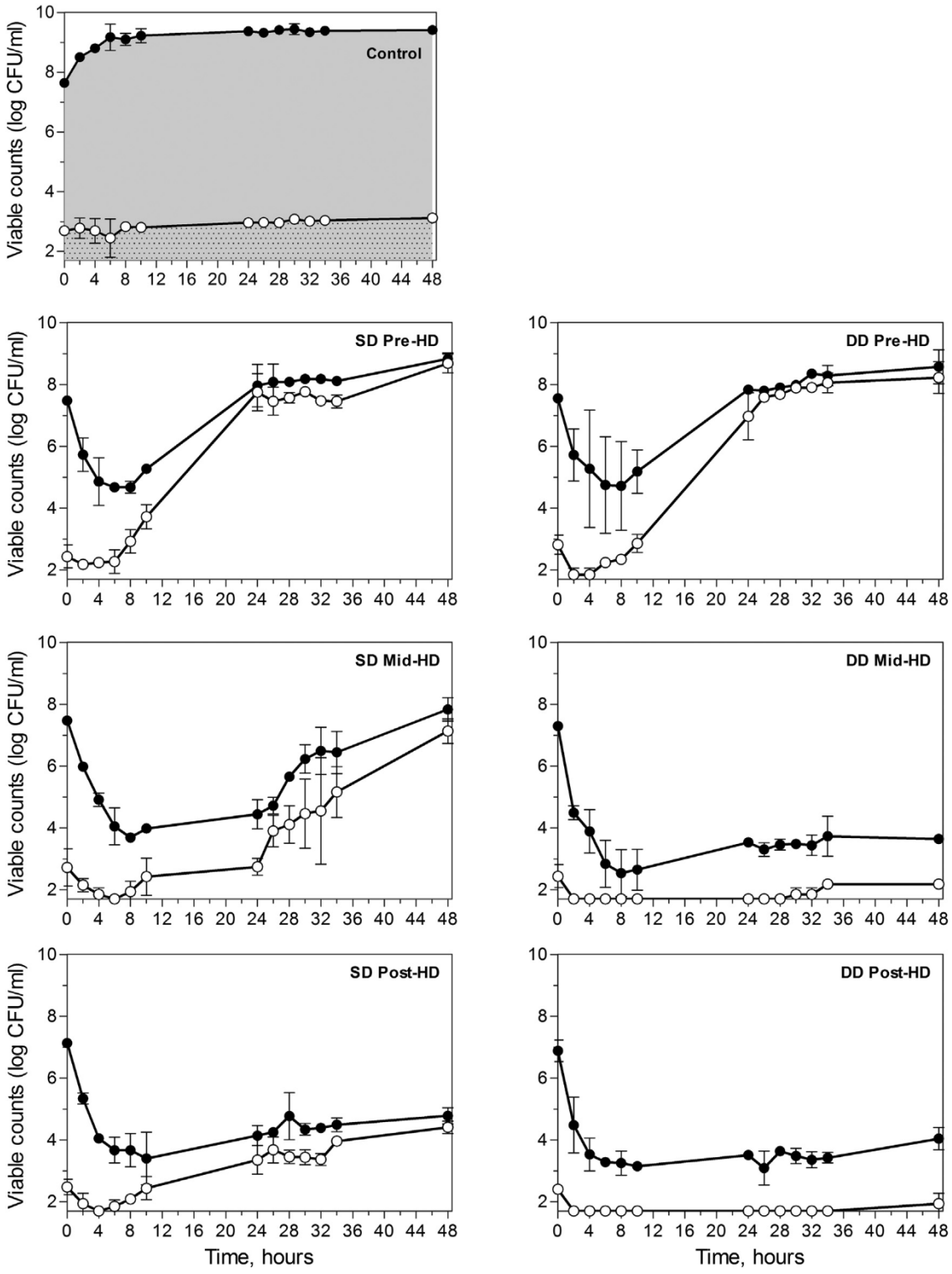

Fig. 4. Bacterial counts (log_{10} CFU/mL) over 48 h obtained using the different amikacin administration schedules with the two doses tested for strain 2 (ST405): bacterial counts over time for the total population (grown in antibiotic-free plates, ●) and the resistant subpopulation (grown in plates containing 3 × MIC of amikacin, ○). As an example, the AUBKC (shaded area) and $AUBKC_m$ (dotted area) are represented in control curves. ST, sequence type; MIC, minimum inhibitory concentration; AUBKC, area under the bacterial killing curve for the total population; $AUBKC_m$, area under the bacterial killing curve for the resistant subpopulations; SD, standard dose, DD, double dose; HD, haemodialysis.

inadequate for infections caused by strains presenting resistant subpopulations [37]. In the last decades, efforts were aimed to establish target PK/PD values as the basis of regimens able to counter the emergence of resistance [37–40].

In the present study, use of three isolates exhibiting the same MIC as well as a study design aimed at achieving the same C_{max} value in the simulations of the three dosing schedules implied that PK/PD parameters based on C_{max} or MIC could not be

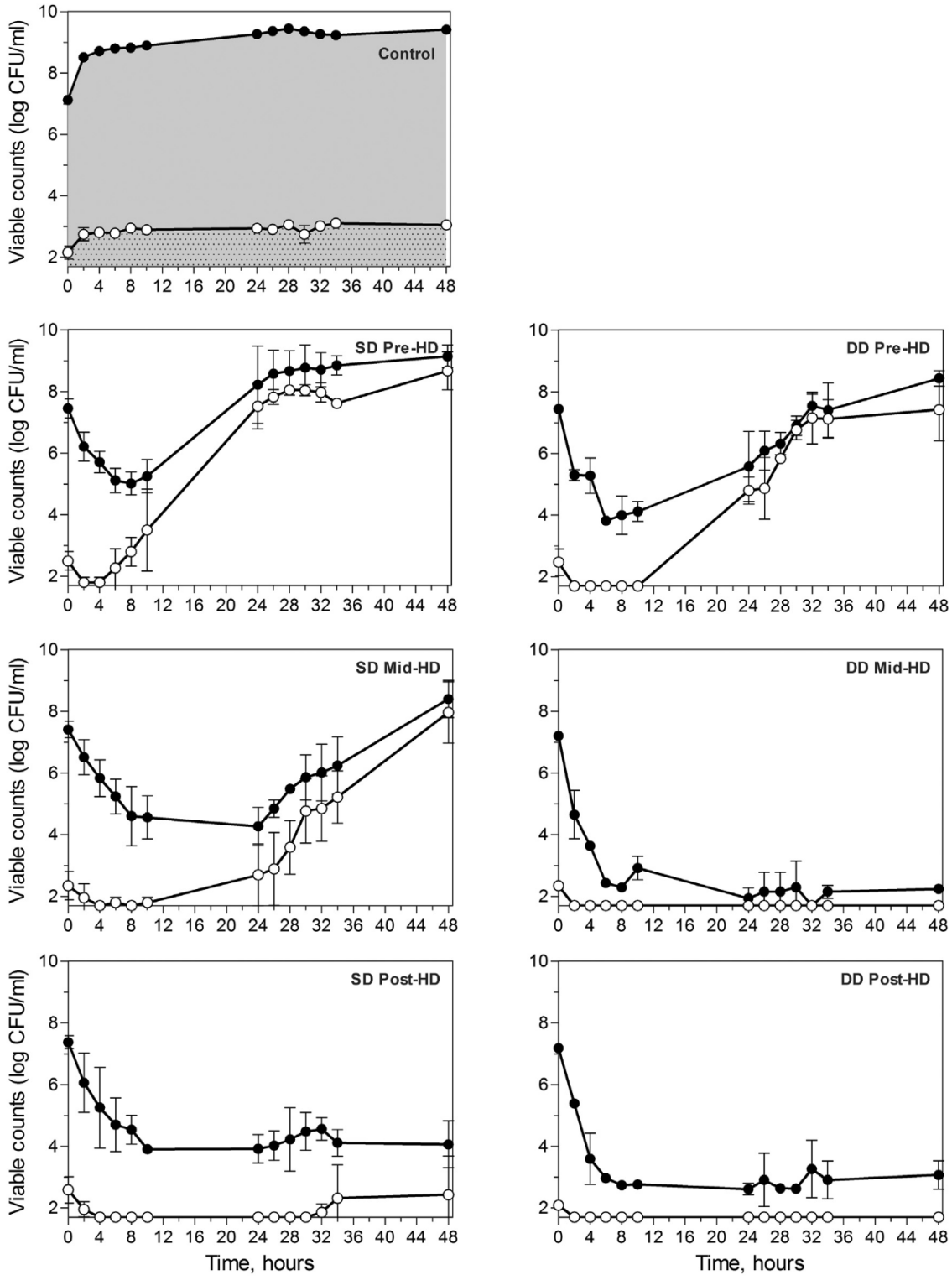

Fig. 5. Bacterial counts (log_{10} CFU/mL) over 48 h obtained using the different amikacin administration schedules with the two doses tested for strain 3 (ST11): bacterial counts over time for the total population (grown in antibiotic-free plates, ●) and the resistant subpopulation (grown in plates containing 3 × MIC of amikacin, ○). As an example, the AUBKC (shaded area) and $AUBKC_m$ (dotted area) are represented in control curves. ST, sequence type; MIC, minimum inhibitory concentration; AUBKC, area under the bacterial killing curve for the total population; $AUBKC_m$, area under the bacterial killing curve for the resistant subpopulations; SD, standard dose, DD, double dose; HD, haemodialysis.

strain-independent predictors of antibacterial activity considering the different behaviour of the three strains along the antibiotic exposure. In this situation, MPC values (different for the three strains) acquired importance for dosing targets [38]. In the current study, regardless of the similarity of MPC/MIC values (7.4–9.7) for the three strains, the estimated values predicting low enrichment of the resistant subpopulations showed low interstrain variability in the case of the AUC_{0-48h}/MPC in contrast to AUC_{0-48h}/MIC where

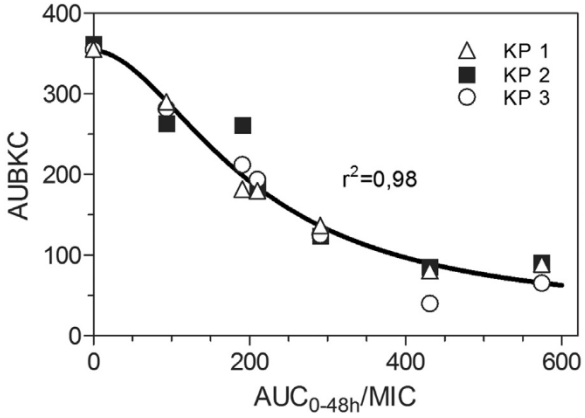

Fig. 6. $AUC_{0–48h}$/MIC relationship with amikacin effect on total (susceptible) population of *Klebsiella pneumoniae* (coefficients of Eq. (1); $y_0 = 23.6$, $y_{max} = 353.7$, $x_{50} = 204.1$ and $b = 1.9$). $AUC_{0–48h}$, area under the concentration–time curve from 0 to 48 h; MIC, minimum inhibitory concentration; AUBKC, area under the bacterial killing curve for the total population.

variability was high. Due to this, the $AUC_{0–48h}$/MPC correlated better with the prevention of growth of resistant subpopulations. The higher predictive value of $AUC_{0–48h}$/MPC was demonstrated with non-aminoglycosides compounds and different bacterial species in some studies [38,41], but not in others [42–44], with none of them linked to situations of haemodialysis.

The MSW hypothesis defines that selection of antibiotic resistant mutants occurs within a concentration range between the MIC and MPC [39]. In the present study, the $\%T_{MSW}$ ($T_{>MPC} > 0$) was strongly correlated with enrichment of resistance, although we could not assess interstrain variability owing to lack of data. The $\%T_{MSW}$ does not consider the position of simulated concentrations within the MSW, which may influence the amplification of resistant mutants [45]. With fluoroquinolones, enrichment of the population with resistant mutants depends not only on the $\%T_{MSW}$ but also on the position of antibiotic concentrations within the MSW, being more pronounced when concentrations are closer to the lower limit, suggesting that $\%T_{MSW}$-based predictions of resistance are less accurate than those based on $AUC_{0–24h}$/MPC [35]. The importance of the position within the MSW was clearly observed for strain 2 (see SD amikacin schedules) where marked differences in enrichment of resistant subpopulations were observed between pre-, mid- and post-HD schedules for the SD with identical $\%T_{MSW}$ values (Table 2). The position within the MSW was also determinant in this study for strains 1 and 3 and the post-HD administration of the SD since, although simulated $\%T_{MSW}$ values (73–76%) were, according to the model, markedly higher than those preventing enrichment of resistant subpopulations (approximately <62%), the fact that concentrations within the MSW were maintained closer to the upper MSW value resulted in a virtually undetectable enrichment of these subpopulations. Due to this, the $AUC_{0–48h}$/MPC was better than the $\%T_{MSW}$ ($T_{>MPC} > 0$) as a predictive parameter in the specific setting of this study, with values of ≈40 being associated with countering of increases in resistant subpopulations. Therefore, the current results are in accordance with the greater antimutant potential of concentrations above the MPC despite the same $\%T_{MSW}$, as previously concluded [35]. Thus, the highest possible dose and haemodialysis ≥24 h after infusion would be necessary to achieve the highest $AUC_{0–48h}$/MPC values able to counter enrichment of resistant subpopulations.

Several limitations of this study can be identified. The strains used in this study represented isolates from an epidemic outbreak

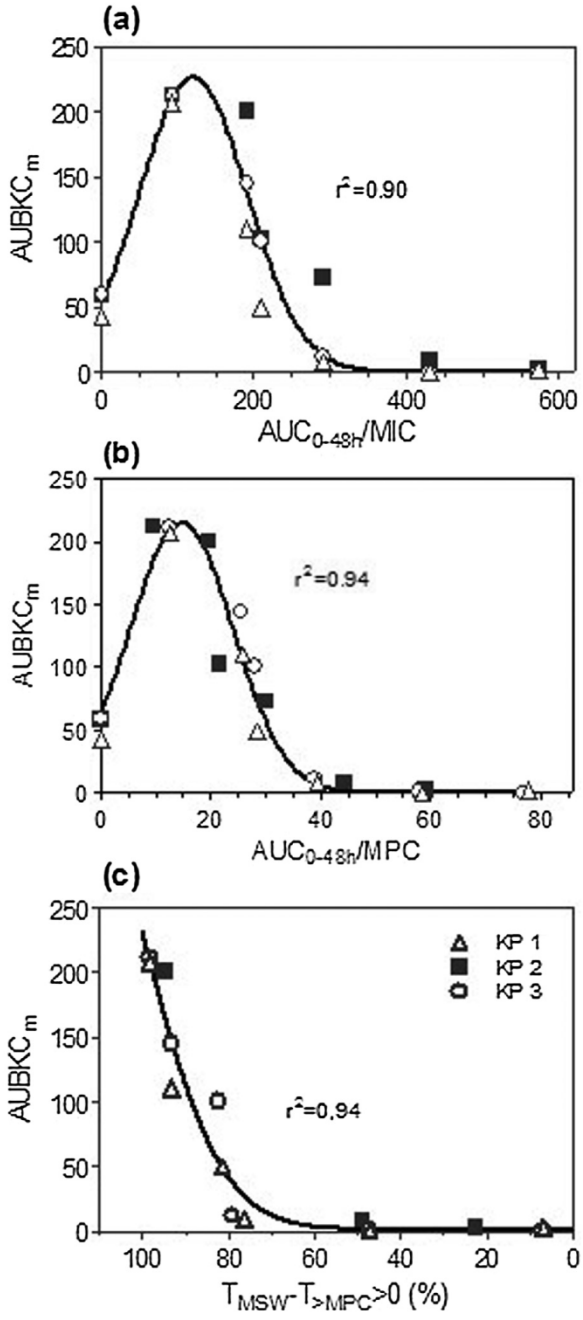

Fig. 7. Correlation of pharmacokinetic/pharmacodynamic indices with resistance in *Klebsiella pneumoniae*: (a): $AUC_{0–48h}$/MIC relationship with $AUBKC_m$ fitted by Eq. (2), $x_m = 120.4$, $a = 226.4$ and $b = 71.1$; (b) $AUC_{0–48h}$/MPC relationship with $AUBKC_m$ fitted by Eq. (2), $x_m = 14.7$, $a = 215.4$ and $b = 9.4$; and (c) $\%T_{MSW}$ ($T_{>MPC} > 0$) relationship with $AUBKC_m$ fitted by Eq. (1), $y_0 = 513.0$, $y_{max} = 0.8$, $x_{50} = 99.8$ and $b = 9.9$. $AUC_{0–48h}$, area under the concentration–time curve from 0 to 48 h; MIC, minimum inhibitory concentration; $AUBKC_m$, area under the bacterial killing curve for the resistant subpopulations; MPC, mutant prevention concentration; $\%T_{MSW}$, percentage of time that the concentration was inside the mutant selection window.

and the results could not be directly extrapolated to other strains/species/resistance determinants. The daily drug exposure (i.e. AUC/MIC) as well as the duration of treatment can contribute to the development of antimicrobial resistance [46] and therefore the short duration of this study may be a limitation. Pre-HD, mid-HD and post-HD amikacin profiles were estimated and were not based on patient data owing to the lack of published data in this specific setting. Finally, the DD was studied to have the highest values of

Table 3
Values of AUC_{0-48h}/MIC and AUC_{0-48h}/MPC that prevented the enrichment of resistant subpopulations according to the model performed by relating experimental data of these parameters with AUBKC.

Strain	AUC_{0-48h}/MIC	AUC_{0-48h}/MPC
1	287.3	38.9
2	371.0	38.2
3	325.4	43.5
All strains	327.8 ± 42.1	40.2 ± 2.9

AUC_{0-48h}, area under the concentration–time curve from 0 to 48 h; MIC, minimum inhibitory concentration; MPC, mutant prevention concentration; AUBKC, area under the bacterial killing curve for the total population.

PK/PD parameters (based on AUC_{0-48h}) without considerations of toxicity in this in vitro study.

5. Conclusion

This study explored PK/PD relationships by simulating amikacin administration in severely ill patients undergoing dialysis facing a MDR epidemic nosocomial outbreak by OXA48KP isolates exhibiting an amikacin MIC at the limit of susceptibility. As classically reported, the highest the AUC_{0-48h}/MIC values, the highest the decreases in total bacterial load. The analysis of PK/PD parameters predicting enrichment of resistance (AUC_{0-48h}/MPC and/or $\%T_{MSW}$) showed that the longest times concentrations were above the MPC (i.e. lowest T_{MSW}), the lowest enrichment of resistant subpopulations. This implies the use of the highest possible dose of amikacin (limited by toxicity) and the post-HD as the best schedule for administration.

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Competing interests

None declared.

Ethical approval

Not required.

References

[1] Paño-Pardo JR, Ruiz-Carrascoso G, Navarro-San Francisco C, Gómez-Gil R, Mora-Rillo M, Romero-Gómez MP, et al. Infections caused by OXA-48-producing *Klebsiella pneumoniae* in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak. J Antimicrob Chemother 2013;68:89–96.
[2] Wesselink JJ, López-Camacho E, de la Peña S, Ramos-Ruiz R, Ruiz-Carrascoso G, Lusa-Bernal S, et al. Genome sequence of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* KpO3210. J Bacteriol 2012;194:6981.
[3] Rodríguez Martínez JM, Díaz-de Alba P, Lopez-Cerero, Ruiz-Carrascoso G, Gomez-Gil R, Pascual A. Presence of quinolone resistance to *qnrB1* genes and bla_{OXA-48} carbapenemase in clinical isolates of *Klebsiella pneumoniae* in Spain. Enferm Infecc Microbiol Clin 2014;32:441–2.
[4] Arana DM, Saez D, García-Hierro P, Bautista V, Fernández-Romero S, Ángel de la Cal M, et al. Concurrent interspecies and clonal dissemination of OXA-48 carbapenemase. Clin Microbiol Infect 2015;21: 148.e1–4.
[5] Argente M, Miró E, Martí C, Vilamala A, Alonso-Tarrés C, Ballester F, et al. Molecular characterization of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* strains after a carbapenem resistance increase in Catalonia. Enferm Infecc Microbiol Clin 2019;37:82–8.
[6] Madueño A, González García J, Fernández-Romero S, Oteo J, Lecuona M. Dissemination and clinical implications of multidrug-resistant *Klebsiella pneumoniae* isolates producing OXA-48 in a Spanish hospital. J Hosp Infect 2017;96:116–22.
[7] Brañas P, Villa J, Viedma E, Mingorance J, Orellana MA, Chaves F. Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital in Madrid: successful establishment of an OXA-48 ST11 clone. Int J Antimicrob Agents 2015;46:111–6.
[8] Villa J, Viedma E, Brañas P, Mingorance J, Chaves F. Draft whole-genome sequence of OXA-48-producing multidrug-resistant *Klebsiella pneumoniae* KP_ST11_OXA-48. Genome Announc 2014;2:e00737–14.
[9] Vasudevan A, Mukhopadhyay A, Li J, Yuen EG, Tambyah PA. A prediction tool for nosocomial multi-drug resistant Gram-negative bacilli infections in critically ill patients—prospective observational study. BMC Infect Dis 2014;14:615.
[10] Schweiger A, Trevino S, Marschall J. Nosocomial infections in dialysis access. Contrib Nephrol 2015;184:205–21.
[11] Roger C, Wallis SC, Muller L, Saissi G, Lipman J, Lefrant JY, et al. Influence of renal replacement modalities on amikacin population pharmacokinetics in critically ill patients on continuous renal replacement therapy. Antimicrob Agents Chemother 2016;60:4901–9.
[12] Moore RD, Smith CR, Lietman PS. Association of aminoglycoside plasma levels with therapeutic outcome in Gram-negative pneumonia. Am J Med 1984;77: 657–62.
[13] Craig WA, Redington J, Ebert SC. Pharmacodynamics of amikacin in vitro and in mouse thigh and lung infections. J Antimicrob Chemother 1991;27(Suppl. C):29–40.
[14] Rybak MJ, Abate BJ, Kang SL, Ruffing MJ, Lerner SA, Drusano GL. Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. Antimicrob Agents Chemother 1999;43:1549–55.
[15] Smyth B, Jones C, Saunders J. Prescribing for patients on dialysis. Aust Prescr 2016;39:21–4.
[16] Berman S. Infections in patients undergoing chronic dialysis. Antimicrobe.org. http:www.antimicrobe.org/e41.asp. [Accessed 8 October 2019].
[17] Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—seventh edition CLSI document M07-A7. Wayne, PA: CLSI; 2006.
[18] Clinical and Laboratory Standards Institute (CLSI). Methods for determining bactericidal activity of antimicrobial agents: approved guideline CLSI document M26-A. Wayne, PA: CLSI; 1999.
[19] Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutant bacteria: measurement and potential use of the mutant selection window. J Infect Dis 2002;185:561–5.
[20] Madhavan T, Yaremchuk K, Levin N, Pohlod D, Burch K, Fisher E, et al. Effect of renal failure and dialysis on the serum concentration of the aminoglycoside amikacin. Antimicrob Agents Chemother 1976;10:464–6.
[21] Regeur L, Colding H, Jensen H, Kampmann JP. Pharmacokinetics of amikacin during hemodialysis and peritoneal dialysis. Antimicrob Agents Chemother 1977;11:214–8.
[22] Matsuo H, Hayashi J, Ono K, Andoh K, Andoh Y, Sano Y, et al. Administration of aminoglycosides to hemodialysis patients immediately before dialysis: a new dosing modality. Antimicrob Agents Chemother 1997;41:2597–601.
[23] Mohamed O, Wahba IM, Earle SB, Bennett WM, Ayres JW, Munar MY. Administration of tobramycin in the beginning of the hemodialysis session: a novel intradialytic dosing regimen. Clin J Am Soc Nephrol 2007;2:694–9.
[24] Zazo H, Martín-Suárez A, Lanao JM. Evaluating amikacin dosage regimens in intensive care unit patients: a pharmacokinetic/pharmacodynamic analysis using Monte Carlo simulation. Int J Antimicrob Agents 2013;42:155–60.
[25] O'Shea S, Duffull S, Johnson DW. Aminoglycosides in hemodialysis patients: is the current practice of post dialysis dosing appropriate? Semin Dial 2009;22:225–30.
[26] Dang L, Duffull S. Development of a semimechanistic model to describe the pharmacokinetics of gentamicin in patients receiving hemodialysis. J Clin Pharmacol 2006;46:662–73.
[27] Bressolle F, Gouby A, Martinez JM, Joubert P, Saissi G, Guillaud R, et al. Population pharmacokinetics of amikacin in critically ill patients. Antimicrob Agents Chemother 1996;40:1682–9.
[28] Kwee F, Andany N, Perl J, Leung E. Open forum. Infect Dis 2016;3(Suppl. 1): S515.
[29] Garraffo R, Drugeon HB, Dellamonica P, Bernard E, Lapalus P. Determination of optimal dosage regimen for amikacin in healthy volunteers by study of pharmacokinetics and bactericidal activity. Antimicrob Agents Chemother 1990;34:614–21.
[30] Jenkins A, Thomson AH, Brown NM, Semple Y, Sluman C, MacGowan A, et al. Amikacin use and therapeutic drug monitoring in adults: do dose regimens and drug exposures affect either outcome or adverse events? A systematic review. J Antimicrob Chemother 2016;71:2754–9.
[31] Gonzalez N, Sevillano D, Alou L, Cafini F, Gimenez MJ, Gomez-Lus ML, et al. Influence of the MBC/MIC ratio on the antibacterial activity of vancomycin versus linezolid against methicillin-resistant *Staphylococcus aureus* isolates in a pharmacodynamic model simulating serum and soft tissue interstitial fluid concentrations reported in diabetic patients. J Antimicrob Chemother 2013;68:2291–5.
[32] Sevillano D, Aguilar L, Alou L, Gimenez MJ, Gonzalez N, Torrico M, et al. Exposure–response analysis of tigecycline in pharmacodynamic simulations using different size inocula of target bacteria. Int J Antimicrob Agents 2010;36:137–44.

[33] Andrews JM. Microbiological assays. In: Reeves DS, Wise R, Andrews JM, White LO, editors. Clinical antimicrobial assays. 1st ed. Oxford, UK: Oxford University Press; 1999. p. 35–44.
[34] González N, Aguilar L, Alou L, Gimenez MJ, Sevillano D, Torrico M, et al. Influence of different resistance traits on the competitive growth of *Haemophilus influenzae* in antibiotic-free medium and selection of resistant populations by different β-lactams: an in vitro pharmacodynamic approach. J Antimicrob Chemother 2009;63:1215–22.
[35] Firsov AA, Lubenko IY, Smirnova MV, Strukova EN, Zinner SH. Enrichment of fluoroquinolone-resistant *Staphylococcus aureus:* oscillating ciprofloxacin concentrations simulated at the upper and lower portions of the mutant selection window. Antimicrob Agents Chemother 2008;52:1924–8.
[36] Zelenitsky SA, Harding GK, Sun S, Ubhi K, Ariano RE. Treatment and outcome of *Pseudomonas aeruginosa* bacteraemia: an antibiotic pharmacodynamic analysis. J Antimicrob Chemother 2003;52:668–74.
[37] Drusano GL, Louie A, Deziel M, Gumbo T. The crisis of resistance: identifying drug exposures to suppress amplification of resistant mutant subpopulations. Clin Infect Dis 2006;42:525–32.
[38] Liang B, Bai N, Cai Y, Wang R, Drlica K, Zhao X. Mutant prevention concentration-based pharmacokinetic/pharmacodynamic indices as dosing targets for suppressing the enrichment of levofloxacin-resistant subpopulations of *Staphylococcus aureus*. Antimicrob Agents Chemother 2011;55:2409–12.
[39] Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin Infect Dis 2001;33 (Suppl. 3):S147–56.
[40] Firsov AA, Portnoy YA, Strukova EN, Shlykova DS, Zinner SH. Predicting bacterial resistance using the time inside the mutant selection window: possibilities and limitations. Int J Antimicrob Agents 2014;44:301–5.
[41] Olofsson SK, Marcusson LL, Komp Lindgren P, Hughes D, Cars O. Selection of ciprofloxacin resistance in *Escherichia coli* in an in vitro kinetic model: relation between drug exposure and mutant prevention concentration. J Antimicrob Chemother 2006;57:1116–21.
[42] Strukova EN, Portnoy YA, Romanov AV, Edelstein MV, Zinner SH, Firsov AA. Searching for the optimal predictor of ciprofloxacin resistance in *Klebsiella pneumoniae* by using in vitro dynamic models. Antimicrob Agents Chemother 2015;60:1208–15.
[43] Strukova EN, Portnoy YA, Zinner SH, Firsov AA. Predictors of bacterial resistance using in vitro dynamic models: area under the concentration–time curve related to either the minimum inhibitory or mutant prevention antibiotic concentration. J Antimicrob Chemother 2016;71:678–84.
[44] Firsov AA, Strukova EN, Shlykova DS, Portnoy YA, Kozyreva VK, Edelstein MV, et al. Bacterial resistance studies using in vitro dynamic models: the predictive power of the mutant prevention and minimum inhibitory antibiotic concentrations. Antimicrob Agents Chemother 2013;57:4956–62.
[45] Zhou J, Dong Y, Zhao X, Lee S, Amin A, Ramaswamy S, et al. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fluoroquinolone-resistant mutations. J Infect Dis 2000;182:517–25.
[46] Tam VH, Louie A, Fritsche TR, Deziel M, Liu W, Brown DL, et al. Impact of drug-exposure intensity and duration of therapy on the emergence of *Staphylococcus aureus* resistance to a quinolone antimicrobial. J Infect Dis 2007;195:1818–27.