


Individualising Therapy to Minimize Bacterial Multidrug Resistance

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Abstract The scourge of antibiotic resistance threatens modern healthcare delivery. A contributing factor to this significant issue may be antibiotic dosing, whereby standard antibiotic regimens are unable to suppress the emergence of antibiotic resistance. This article aims to review the role of pharmacokinetic and pharmacodynamic (PK/PD) measures for optimising antibiotic therapy to minimise resistance emergence. It also seeks to describe the utility of combination antibiotic therapy for suppression of resistance and summarise the role of biomarkers in individualising antibiotic therapy. Scientific journals indexed in PubMed and Web of Science were searched to identify relevant articles and summarise existing evidence. Studies suggest that optimising antibiotic dosing to attain defined PK/PD ratios may limit the emergence of resistance. A maximum aminoglycoside concentration to minimum inhibitory concentration (MIC) ratio of > 20 , a fluoroquinolone area under the concentration–time curve to MIC ratio of > 285 and a β -lactam trough concentration of

$> 6 \times \text{MIC}$ are likely required for resistance suppression. In vitro studies demonstrate a clear advantage for some antibiotic combinations. However, clinical evidence is limited, suggesting that the use of combination regimens should be assessed on an individual patient basis. Biomarkers, such as procalcitonin, may help to individualise and reduce the duration of antibiotic treatment, which may minimise antibiotic resistance emergence during therapy. Future studies should translate laboratory-based studies into clinical trials and validate the appropriate clinical PK/PD predictors required for resistance suppression in vivo. Other adjunct strategies, such as biomarker-guided therapy or the use of antibiotic combinations require further investigation.

Key Points

Antibiotic doses for suppression of emergence of resistance are likely to exceed doses approved for treatment.

Antibiotic combination therapy may be one method to minimise resistance emergence.

The role of biomarkers to reduce resistance emergence is yet to be determined.

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1 Introduction

Antibiotic resistance is a significant global health issue, particularly considering the association of increased mortality and length of hospital admission for patients with multi-drug-resistant (MDR) bacterial infections [1, 2]. The global spread of bacterial resistance to last-line antibiotics such as carbapenems and colistin threatens the return of a pre-antibiotic era [3, 4]. This issue is likely to further escalate given the lack of novel antibacterial drug classes that have been developed in recent decades that can treat infections caused by MDR pathogens [5]. Given that the drug development process may take decades, optimising the use of currently available antibiotics should be considered an essential tool to minimise antibiotic resistance emergence.

Methods to maximise the utility of currently available antibiotics include the optimisation of the dose and duration of treatment, along with the use of combination therapies, which may limit resistance emergence and potentially treat MDR or extensively drug-resistant bacterial infections [6, 7]. Optimisation of antibiotic dosing can be guided by pharmacokinetic and pharmacodynamic (PK/PD) ratios that relate the antibiotic exposure to improved clinical outcomes. Dosing that aims to suppress resistance may also be potentially guided by PK/PD ratios. In vitro and pre-clinical in vivo studies have described PK/PD ratios required to prevent antibiotic resistance emergence. However, exposures required for suppression of emergence of resistance are generally higher than that needed for clinical cure, suggesting that current dosing practices are unlikely to ensure the prevention of antibiotic resistance emergence [8]. A further measure to prevent resistance emergence may include minimising the duration of therapy. As clinical signs and symptoms of infection may persist for some time after bacterial eradication has occurred, biomarkers may enable the distinction between those who remain ill due to persistent bacterial infection and those who remain symptomatic after bacterial eradication [9]. Thus, biomarkers may permit reduced antibiotic use and limit antibiotic selective pressure [10].

This article aims to describe the use of PK/PD measures for the optimisation of antibiotic therapy with the goal of minimising resistance emergence. It also aims to summarise existing evidence regarding the role of combination antibiotic therapy for suppression of resistance. The use of biomarkers to guide clinical decision making with respect to limiting the duration of antibiotic therapy and its role in minimising resistance emergence is also discussed.

2 Methods

Published studies from 1946 until August 2017 were identified by searching the PubMed and Web of Science databases. Additional references were obtained by a bibliography and citation search of previously identified articles. Combinations of the following search terms were used: antibiotic, sepsis, septic shock, pharmacokinetic, pharmacodynamic, biomarker, resistant, bacteria and combination. Only studies written in English were reviewed. Any study describing antibiotic PK/PD ratios, the use of biomarkers to individualise therapy and antibiotic combination therapy, focussing on the emergence of resistance, were included.

3 Pharmacokinetic/Pharmacodynamic (PK/PD) Measures for Individualisation of Antibiotic Dosing

No objective and easily assessable clinical endpoint is available to guide antibiotic dose titration in a timely manner. The lack of an easily assessable and available clinical endpoint to ensure treatment adequacy and infection resolution (the ultimate goal of therapy) has led to the use of surrogate PK/PD ratios to guide both the design and the individualisation of antibiotic dosing. PK/PD ratios relate antibiotic exposure, usually relative to the minimum inhibitory concentration (MIC) of the pathogen with either bacterial killing or patient clinical outcomes [11]. Adjusting antibiotic dosing regimens to achieve these ratios can increase the likelihood that a patient will achieve desired outcomes [12].

The appropriate dosing adjustment strategy depends on the type of PK/PD ratio that best describes the clinical efficacy and bacterial kill characteristics of the antibiotic [13]. For concentration-dependent antibiotics, such as aminoglycosides, the preferred PK/PD ratio relates the free-drug maximum concentration (fC_{max}) to the MIC (fC_{max}/MIC) [14]. For time-dependent antibiotics such as β -lactam antibiotics, antibacterial effect is best described by the percentage of time the free drug concentration remains above the MIC throughout the dosing interval ($fT_{>MIC}$) [15, 16]. On the other hand, the best PK/PD ratio for drugs with a more mixed concentration and time dependence, such as vancomycin, relate the area under the free-drug concentration–time curve, typically over a 24 h period, to the MIC ($fAUC_{0-24}/MIC$) (Fig. 1 and Table 1) [8, 17]. These conventional PK/PD ratios have also been related to suppression of emergence of resistance (Fig. 1) although, as for clinical efficacy, the relationships are largely based on pre-clinical studies with a

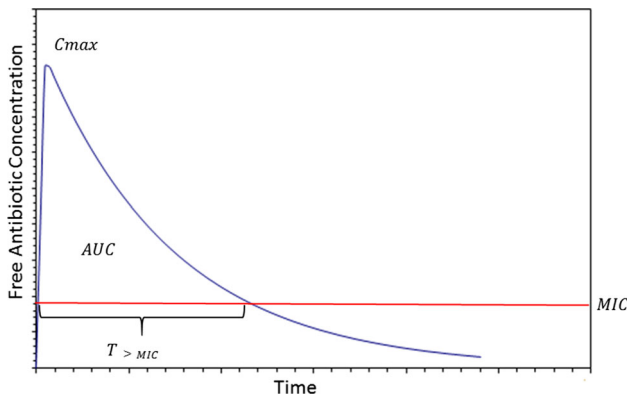


Fig. 1 Pharmacokinetic and pharmacodynamic parameters that define pharmacokinetic/pharmacodynamic ratios. *AUC* area under the concentration–time curve, *C_{max}* maximum (peak) concentration, *MIC* minimum inhibitory concentration, *T_{>MIC}* percentage of time the free drug concentration remains above the MIC throughout the dosing interval

limited number of bacterial isolates. Prospective clinical trials are required to validate the PK/PD targets derived from in vitro studies.

In addition to the use of conventional PK/PD ratios that are based on the MIC of the pathogen, previous works have suggested that dosing aiming to suppress the emergence of resistance should preferentially be based on the mutant prevention concentration (MPC) [48, 49]. The MPC is derived from in vitro observation that the growth of resistant organisms can occur at a concentration above the MIC, but is inhibited at a threshold concentration defined as the MPC [34, 35]. Per the MPC definition, at least one mutation is needed for an isolate to grow in the presence of an antibiotic at a concentration above the MIC; however, two or more mutations conferring antibiotic resistance are required to grow at a concentration exceeding the MPC [34, 35]. Therefore, PK/PD ratios based on the MPC may be an appealing antibiotic dosing target as the probability of an organism attaining two or more mutations conferring antibiotic resistance is unlikely [34, 35]. Thus, when compared against increasing antibiotic concentrations, the emergence of resistance is typically described by an inverted ‘U’ shape (Fig. 2), where concentrations between the MIC and the MPC, known as the mutant selection window (MSW), promote the growth of a resistant bacterial subpopulation. This suggests the dosing regimen

Table 1 Pharmacokinetic/pharmacodynamic ratios identified for efficacy and resistance suppression

Antibiotic class	Type of PK/PD ratio and target value for clinical efficacy			Type of PK/PD ratio and target value for resistance suppression		
	PK/PD ratio	Target value	References	PK/PD ratio	Target value	References
Aminoglycoside	<i>C_{max}</i> /MIC	> 10	[18, 19]	<i>C_{max}</i> /MIC	> 20	[20]
	<i>AUC</i> /MIC	> 70	[21, 22]			
Penicillins	<i>fT_{>MIC}</i>	> 50%	[17, 23, 24]	<i>C_{min}</i> /MIC ^a	> 4.6	[25]
				<i>fT_{>MIC}</i> ^b	> 100%	[26]
Cephalosporins	<i>fT_{>MIC}</i>	> 70%	[17, 23, 24]	<i>C_{min}</i> /MIC ^a	> 3.8	[27]
Carbapenems	<i>fT_{>MIC}</i>	> 40%	[17, 23, 24]	<i>C_{min}</i> /MIC ^a	> 6.2	[28]
				<i>T_{MSW}</i>	< 20%	[29]
Fluoroquinolones	<i>AUC</i> /MIC	> 125	[30, 31] ^a	<i>AUC</i> /MIC ^a	> 285	[32]
	<i>fAUC</i> /MIC	> 33.7	[33] ^b	<i>AUC</i> /MIC ^b	> 143	[34, 35]
	<i>C_{max}</i> /MIC	> 8	[21]	<i>AUC</i> /MPC	> 22	[36]
			<i>T_{MSW}</i>	< 20%	[35]	
Vancomycin	<i>AUC</i> /MIC	> 400	[37] ^b	<i>AUC</i> /MIC	> 200	[38, 39] ^b
Linezolid	<i>AUC</i> /MIC	> 80	[40, 41]	<i>AUC</i> /MIC	31–495	[42, 43] ^b
	<i>fT_{>MIC}</i>	> 82.1%	[40]			
Daptomycin	<i>AUC</i> /MIC	388–537	[44] ^c	<i>AUC</i> /MIC	> 200	[38]
Colistin	<i>AUC</i> /MIC	> 50	[45] ^c	NA		
Fosfomycin	<i>AUC</i> /MIC	> 8.6	[46] ^c	<i>AUC</i> /MIC	> 3136	[47] ^b

AUC area under the concentration–time curve over 24 h, *C_{max}* maximum concentration during a dosing interval, *C_{min}* minimum concentration during a dosing interval, *f* free concentration, *MPC* mutant prevention concentration, *NA* no dose studied has effectively suppressed resistance emergence, *PK/PD* pharmacokinetics/pharmacodynamics, *T_{>MIC}* time that the concentration is maintained above the minimum inhibitory concentration of the bacteria, *T_{MSW}* time that the antibiotic concentration remains within the mutant selection window

^aAgainst Gram-negative organisms

^bAgainst Gram-positive organisms

^cBased on animal models of bactericidal activity

required for suppressing the emergence of antibiotic resistance is greater than that required for clinical cure. The MPC has been extensively described for fluoroquinolones [34–36, 49, 50] and to some extent for β -lactams [48, 51], aminoglycosides [52] and macrolides [53]. However, routine measurement of the MPC for use as a dosing target is not yet warranted as PK/PD ratios incorporating the MPC are uncommon and present a number of additional issues that require consideration. First, antibiotic exposure within the MSW is likely adequate for clinical cure in immunocompetent patients as the remaining bacterial burden is eradicated by the immune response [54, 55]. Second, the MPC reflects activity of the antibiotic against existing resistant subpopulations in vitro and not necessarily against acquired resistance mechanisms in vivo [56], although limited evidence suggests that the MPC may be independent of the resistance mechanism [57]. Third, the PK/PD ratios based on the MPC required to suppress resistance are not yet well described. Finally, the MPC requires additional culturing time of up to 72 h compared with up to 20 h for a standard MIC test [58] or between 5 and 19 h for Vitek2 MIC methods [59]. Nonetheless, the potential utility of the MPC as a guide for dose individualisation to minimise resistance deserves further investigation. One practical challenge is the relatively high MPC values that may be encountered for some antibiotics with narrow therapeutic windows, such as the aminoglycosides [52] and vancomycin [60]. Given that the MPC is not routinely used

clinically, PK/PD ratios based on the MPC are difficult to derive from clinical studies. In this regard, translational in vitro studies, such as the dynamic hollow fiber infection model (HFIM), which enables the simulation of a human pharmacokinetic (PK) profile, thus potentially allowing extrapolation into clinical use, provides a useful guide for dosing that ensures suppression of resistance emergence [61, 62].

4 What Information is Needed to Individualise Therapy?

4.1 Bacterial Minimum Inhibitory Concentration

PK/PD ratios that guide dose individualisation are currently based on the bacteria MIC. Microbiology laboratories routinely report whether bacteria are susceptible or resistant if the bacteria MIC is below or above the clinical breakpoint cut-off, respectively, but not necessarily the specific pathogen MIC, which may involve a separate assay than that used to determine susceptibility [63]. It has been well established that a ‘susceptible’ pathogen does not equate to the adequacy of antibiotic exposure, particularly for isolates with MICs near the clinical breakpoint (Table 1). This is exemplified by the decrease in estimated probability of attaining a target C_{\max}/MIC of ≥ 10 for amikacin by nearly 60% when the pathogen MIC increases from 4 to 8 mg/l, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility breakpoint for amikacin, even when administered at a dose of 30 mg/kg in patients with ventilator-associated pneumonia (VAP) [64]. Similar trends of reduced probability of target attainment within the susceptibility range have been described for meropenem [65], ciprofloxacin [66] and piperacillin–tazobactam [67].

The reduced probability of target attainment when using conventional doses against susceptible isolates with increased MICs for current clinical PK/PD indices suggests that doses need to be adjusted based on the measured MIC to achieve optimal PK/PD ratios. However, the utility of the MIC is questionable. The MIC is determined in optimal bacterial growth conditions in the presence of static serial twofold increasing antibiotic concentrations, which do not represent the in vivo environment in a patient receiving antibiotics therapeutically [68]. Moreover, the MIC is the observed minimum antibiotic concentration to inhibit bacterial growth. Given that a bacterial suspension is visibly turbid at an inoculum of $\sim 1 \times 10^7$ colony-forming units (CFU)/ml, the MIC may also be interpreted as the concentration of antibiotic that inhibits growth below this bacterial burden [68]. The implication is that the MIC of a bacterial inoculum likely reflects the predominant bacterial

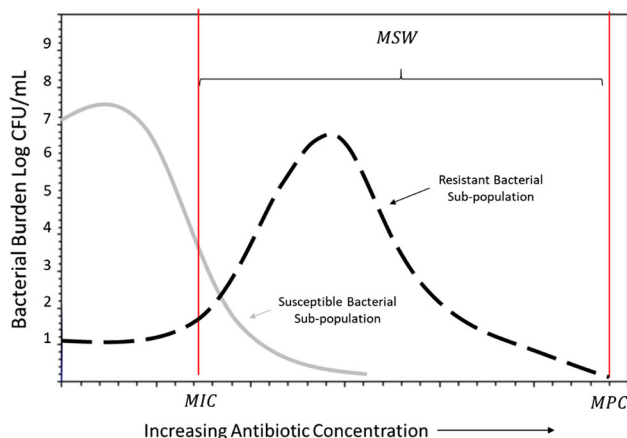


Fig. 2 The effect of exposure to increasing antibiotic concentrations on the burden of resistant and susceptible bacterial populations. The susceptible (grey) bacterial burden is rapidly reduced against an antibiotic concentration equivalent to the minimum inhibitory concentration, which has little impact on the resistant (black) bacterial subpopulation. The resistant bacterial subpopulation killing curve is represented by the ‘inverted U-shape’, where the resistant population is eventually killed at the antibiotic mutant prevention concentration. CFU colony-forming units, MIC minimum inhibitory concentration, MPC mutant prevention concentration, MSW mutation selection window

population and does not consider a potentially 'resistant' or 'less-susceptible' bacterial subpopulation. Therefore, targets based on the MIC for suppression of resistance emergence, although commonly expressed as such, are of uncertain value in determining the actual antibiotic exposure required to suppress resistance emergence as the MIC does not reflect the antibiotic exposure to prevent growth of the 'resistant' bacterial subpopulation in a defined inoculum. Pursuant to this concept is the use of a high starting bacterial inoculum ($\sim 1 \times 10^8$ CFU/ml) in many studies that investigate bacterial resistance emergence, which exceeds that required for MIC testing ($\sim 1 \times 10^{5.5}$ CFU/ml) [58]. It would be expected that as the total bacterial population increases, so too does the probability of a 'resistant' bacterial subpopulation as can be demonstrated by the growth of bacteria in the presence of an antibiotic, typically at concentrations equivalent to a few multiples of the MIC, known as the mutation frequency [69–71]. For example, if we consider a reported fluoroquinolone mutation frequency of $\sim 1 \times 10^{-8}$ for Gram-negative pathogens, this would mean that a resistant subpopulation would be unlikely for a standard MIC test but may result in the emergence of resistance for some patients with high inoculum infections such as VAP [72–76].

To make use of the MIC that describes the antibiotic–pathogen PD, a clinician must also understand how to adjust the antibiotic dosing regimen to optimise PK/PD ratios to ensure optimal clinical efficacy.

4.2 Therapeutic Drug Monitoring and pharmacokinetic Models

Knowledge of the drug PK profile within the patient at the bedside is necessary to enable precise dose individualisation, guided by PK/PD ratios. The PK information in a patient is obtained from antibiotic concentration measurement with the use of therapeutic drug monitoring (TDM) [77]. Critically ill patients in the intensive care unit (ICU) have well documented altered PK related to enhanced or impaired elimination, or increased volume of distribution due to increased vascular permeability because of sepsis [14, 78, 79]. These result in PK fluctuations that may necessitate TDM-guided dose adjustment to maximise the likelihood of achieving therapeutic concentrations. For example, in one study, 74.2% of patients receiving β -lactam antibiotic therapy required a dose increase to meet a minimum plasma concentration (C_{\min})/MIC ratio > 4 [80]. Similarly, amikacin dose adjustment was required in 83% of patients with sepsis, with approximately equivalent rates of dose increases and decreases [81]. The use of TDM to guide therapy has also been described for other antibiotics, including the glycopeptides [82], fluoroquinolones [12], linezolid [83] and daptomycin [84].

TDM with dose adjustment to meet key PK/PD targets has been shown to reduce mortality in patients with nosocomial pneumonia receiving aminoglycosides, β -lactam antibiotics and/or fluoroquinolones [12]. Dose adjustment with PK/PD target attainment of the prescribed antibiotics within 3 days of treatment initiation resulted in a lower mortality rate of 10.2% for the TDM cohort compared with 23.6% for the control cohort, highlighting the importance of dosing adjustment to attain PK/PD measures [12]. Moreover, patients with severe sepsis or septic shock achieving a target amikacin C_{\max} of between 60 and 80 mg/l (corresponding to a C_{\max} /MIC ratio of ~ 8) had reduced mortality compared with those who did not; which has also been shown in the treatment of nosocomial pneumonia [19, 85]. However, when interpreting the MIC result, the clinician should consider the impact of potential MIC assay error, which may be within one dilution of the determined value and thus affect the target drug exposure [68]. The lack of prospective randomised controlled trials supporting PK/PD targeted therapy, timely availability of MIC data, and limited TDM resources for many antibiotics have prevented widespread adoption of PK/PD-guided therapy [86].

A plethora of software packages have been developed to perform Bayesian dose adaptation at the bedside, whereby a single well-timed TDM concentration can be incorporated into a population PK model together with patient-specific clinical covariates to provide a recommended dose that may meet key PK/PD ratios. Several packages have been reviewed [13]. When clearly defined PK/PD ratios associated with resistance suppression are available, dosing software may be adjusted accordingly to predict patient-specific dosing regimens that minimise emergence of resistance.

5 PK/PD Measures to Minimise Resistance Emergence and Importance of Antibiotic PK at the Site of Infection

5.1 Aminoglycosides

PK/PD ratios of aminoglycosides required for suppression of resistance emergence have not been well established. When conventional PK/PD targets are used to guide aminoglycoside dosing, resistance emergence may occur within 12 h of the first dose [87]. Thus, it is generally expected that higher PK/PD ratios than required for clinical effect may be required to suppress resistance. This was demonstrated by Tam et al. [20], who showed, using one-compartment dynamic in vitro studies whereby bacteria were exposed to a humanised drug concentration–time profile, that a twice daily dosing regimen of gentamicin

with both doses targeting a $C_{\max}/\text{MIC} > 30$ against *Pseudomonas aeruginosa* resulted in sustained resistance suppression. In contrast, single daily doses targeting a $C_{\max}/\text{MIC} > 36$ did not reduce resistance emergence. This discrepancy is likely a function of the increased AUC/MIC ratio in the twice daily dosing regimens, which is not surprising given both the AUC/MIC [88] and the C_{\max}/MIC [18] ratio have been correlated with clinical efficacy (Table 1). Therefore, it is important for treatment efficacy and minimisation of toxicity that the shape of the AUC reflects the contribution of the concentration-dependent bacterial killing property of aminoglycosides [88]. However, a once daily dose of amikacin with a $C_{\max}/\text{MIC} > 20$ against *Acinetobacter baumannii* suppressed the emergence of resistance [20]. This inter-isolate and inter-species differences in the required aminoglycoside target concentration for suppressing resistance emergence likely relates to non-specific outer membrane changes given that amikacin resistance against *A. baumannii* was stable with serial passaging; however, *P. aeruginosa* gentamicin resistance was transient [20]. Therefore, the dose required to suppress resistance emergence likely varies with specific drug within an antibiotic class and with the bacterial species or specific isolate. In any case, these results highlight that dosing targets at least twice that required for clinical efficacy may be necessary to ensure an exposure that minimises resistance; however, the risk of significant nephrotoxicity with such regimens likely precludes the implementation into routine practice until the risk–benefit ratio of such dosing regimens have been established [89]. Layeux et al. [90] demonstrated that one potential approach could be the use of high-dose aminoglycoside therapy in combination with dialysis to enable attainment of high C_{\max} and AUC values but to minimise drug accumulation and toxicity through increased extracorporeal clearance.

The ability to meet target PK/PD ratios depends on the patient's site of infection and mode of drug administration. Serum concentrations achieved with intravenous high-dose regimens of gentamicin and tobramycin (7 mg/kg), and amikacin (30 mg/kg) are unlikely to meet the proposed PK/PD ratios for resistance suppression [91, 92]. Even current clinical PK/PD indices for efficacy, which are at least half that required for resistance suppression (Table 1), may not be met in up to 41% of patients [91, 92].

For respiratory infections, nebulised aminoglycoside administration can attain concentrations in the epithelial lining fluid, the proposed site of bacterial deposition in pneumonia, up to 46-fold that of intravenous therapy [93]. This may be one method to minimise resistance emergence while improving treatment outcomes as has been shown in a recent meta-analysis [94]. Aminoglycosides are also commonly used in the management of complicated urinary tract infections (UTIs). Gentamicin achieves peak urinary

concentrations approximately 26-fold that in serum [95], which may achieve proposed PK/PD targets for resistance suppression; however, the impact of this on bacterial eradication and resistance emergence has yet to be determined experimentally. Taken together, these data suggest that the AUC/MIC and the C_{\max}/MIC ratio are likely key PK/PD ratios to consider for resistance suppression, which may be attainable in the urine with modest aminoglycoside doses or through nebulisation for the treatment of respiratory infections.

5.2 Fosfomycin

Fosfomycin has been extensively used as an oral antibiotic for treating UTIs in Europe and is now also being increasingly used intravenously in the management of invasive infections caused by MDR bacteria [96]. Little is known regarding the optimal dosing strategy for clinical cure or resistance suppression; however, murine thigh infection models suggest an AUC/MIC ratio of $> 8.5 \text{ h}^{-1}$ is the minimum exposure required for bacteriostatic activity and mice survival, but this varies between species and bacterial isolate [46]. The AUC/MIC ratio has been linked with the suppression of emergence of resistance. In a dynamic in vitro HFIM simulating an intravenous fosfomycin total daily dose of 24 g, either as a single dose or administered in divided doses against an *Escherichia coli* isolate with a fosfomycin MIC of 1 mg/l suppressed resistance emergence (AUC_{0–24}/MIC of 3136 h^{−1}) [47]. It has also been postulated that the time above the MIC of any resistant subpopulation(s) is important for fosfomycin efficacy and reduction in resistance emergence [97]. When the fosfomycin concentration is above the MIC of the resistant subpopulation for 33% of the dosing interval, the bacterial concentration is reduced by 2-logs; however, the time above the MIC of the resistant subpopulation was not able to be quantified.

Fosfomycin is concentrated in the urine with AUC_{0–∞} of 31,995 mg/l.h following a single oral dose of 3 g, which is likely to exceed the AUC/MIC ratio potentially required for resistance suppression for isolates with an MIC of less than 8 mg/l [98]. Similar findings in a novel in vitro dynamic simulation of bladder fosfomycin concentrations following oral dosing show that a single oral dose of fosfomycin 3 g against *E. coli* and *Enterobacter cloacae* isolates with an MIC ≤ 4 mg/l is likely sufficient to suppress resistance emergence [99]. However, resistance emergence occurred with all *Klebsiella pneumoniae* isolates tested (MIC range 2–8 mg/l) [99].

High urinary tract concentrations, for some isolates, may partly explain the lack of resistance emergence when used for UTIs [100]. These exposures are unlikely to be attained in plasma or interstitial fluid with approved/safe high-dose

oral or intravenous therapy, which may partly explain the rates of resistance emergence during the treatment of systemic infections with monotherapy, which may occur in up to 20% of patients with infections other than a UTI [100–102]. Nebulised fosfomycin for the treatment of pneumonia may be one alternate administration potentially resulting in the required concentrations to minimise resistance emergence [103].

5.3 β -Lactam Antibiotics

β -Lactam antibiotics demonstrate time-dependent bactericidal activity, and the plasma ($\% fT_{>MIC}$) correlates well with clinical outcomes and bactericidal activity [17]. The traditional $fT_{>MIC}$ values recommended for optimal effect are 40, 50 and 60–70% for carbapenems, penicillins and cephalosporins, respectively [17, 23]. However, these proposed ratios are likely insufficient for optimal patient outcomes, and higher PK/PD targets of as much as 100% $fT_{>2-5 \times MIC}$ have been proposed to be required for maximising clinical efficacy [79, 104–107]. Evidence for resistance suppression in clinical trials is scarce. A study in patients with VAP could not identify a statistically significant PK/PD index for carbapenems, based on either the MIC ($fT_{>MIC}$) or the MPC ($fT_{>MPC}$) [108]. However, resistance emergence was greatest (26%) in patients with an $fT_{>MIC}$ of $< 40\%$ [108].

In vitro dynamic infection models and in vivo animal model studies have described PK/PD ratios that may suppress resistance emergence. Meropenem C_{min}/MIC ratios of > 6.2 have been shown in vitro with the dynamic in vitro HFIM as suppressing resistance emergence against *P. aeruginosa* [28]. Interestingly, this ratio was reduced to < 1.7 when meropenem was used in combination with tobramycin [28]. With the dynamic in vitro HFIM, C_{min}/MIC ratios of > 3.8 have also been proposed for cefepime and ceftazidime against *P. aeruginosa* and *K. pneumoniae* isolates [27]. Moreover, should the concentration of meropenem remain within the MSW for $> 80\%$ of the dosing interval, resistance appears to be amplified, which is consistent with the MSW hypothesis [29]. Indeed, attainment of the accepted $fT_{>MIC}$ of 40% for meropenem, the traditional PK/PD ratio recommended for optimal clinical outcomes, was associated with *P. aeruginosa* resistance amplification in a murine pneumonia model, despite providing a 100% mice survival rate compared with 0% for untreated controls [109]. Similar results have been described by Zinner et al. [110] using the dynamic in vitro HFIM with doripenem against *P. aeruginosa*.

Results appear to be similar for the penicillins against Gram-negative bacterial pathogens. Felton et al. [25] described that a piperacillin–tazobactam C_{min}/MIC ratio of 4.6 would be required for resistance suppression with

intermittent bolus dosing against a relatively low bacterial inoculum (4×10^5 CFU/ml) in the dynamic in vitro HFIM. Interestingly, the target C_{min}/MIC ratio is increased with continuous infusion regimens, with a ratio of 10.4 required for resistance suppression [25]. However, with intermittent regimens achieving C_{min}/MIC ratios of 4.6, the plasma concentration to MIC ratio is likely to be greater than 10.4 for a substantial proportion of the dosing interval. Thus, prolonged exposure to concentrations higher than the C_{min} of intermittent dosing may be required for resistance suppression. Practically, compared with continuous infusions, higher doses are required with intermittent regimens to achieve the respective ratios. Furthermore, in the same dynamic in vitro HFIM study, simulated high-dose piperacillin regimens of up to 17 g thrice daily administered as a bolus or extended infusion did not result in significant bacterial killing and was unable to suppress the emergence of resistance against a large *P. aeruginosa* bacterial density ($\sim 8 \times 10^8$ CFU/ml) [25]. Therefore, combination antibiotic therapy may be necessary to treat and suppress the emergence of resistance against infections with a high bacterial burden, such as VAP [72–76]; however, this requires further investigation.

For the newly available β -lactam antibiotic and β -lactamase inhibitor combinations, including ceftolozane–tazobactam and ceftazidime–avibactam, the PK/PD indices for resistance suppression are not yet well described. In the dynamic in vitro HFIM, simulated doses of ceftolozane–tazobactam 2–1 g administered every 8 h (achieving 100% $fT_{>MIC}$) did not result in growth of resistant *P. aeruginosa*, but doses between 125–62.5 mg (achieving 12.5% $fT_{>MIC}$) and 1–0.5 g (achieving 100% $fT_{>MIC}$) were associated with resistance amplification [111]. This discrepancy is likely due to the different ceftolozane AUC between the 2–1 g (AUC_{0–24} 1032.6 mg/l.h) and 1–0.5 g (AUC_{0–24} 456.2 mg/l.h), which would likely result in an elevated C_{min}/MIC ratio that has been associated with suppression of emergence of resistance [27, 111]. In a similar study using *E. coli*, a lower dose threshold of 750–375 mg administered every 8 h (100% $fT_{>MIC}$) resulted in resistance suppression [112]. The difference between these studies is the lower MIC of *E. coli* (0.25 mg/l) against ceftolozane–tazobactam compared with *P. aeruginosa* (4 mg/l). The exposures required of the β -lactamase inhibitor in combination products is currently unknown, but the minimum concentration required for enzyme inhibition is likely important, as shown by the inhibition of AmpC that mediated cefepime resistance emergence in an HFIM study [87]. This concept is further supported by the suppression of bacterial regrowth when exposed to avibactam concentrations > 0.28 mg/l when combined with ceftazidime [113, 114]. Currently used ceftazidime–avibactam doses of up to 2–0.5 g administered intravenously thrice daily in a

cohort of 37 patients with carbapenem-resistant *Enterobacteriaceae* infections have been associated with microbiological failures in up to 27% of patients, with 30% of these failures likely related to the emergence of ceftazidime–avibactam-resistant isolates [115]. Given that treatment was more likely to fail in patients with renal replacement therapy and lung infections, dosing consideration for target site antibiotic penetration [116] and extracorporeal elimination [117] are likely key factors influencing treatment outcomes [115]. This would suggest that dose optimisation must be considered to prevent resistance emergence to newly developed antibiotics.

PK/PD ratios for β -lactams against Gram-positive organisms are lacking, but a $fT_{>MIC}$ of 100% may be sufficient against susceptible *Streptococcus pneumoniae* [26, 118].

Given the lack of data describing significant toxicity with supratherapeutic concentrations of penicillins [119, 120] and potential impact on clinical outcomes, we would advocate the use of an initial loading dose followed by continuous infusions of high-dose β -lactam antibiotics for the treatment of patients with sepsis or septic shock requiring ICU admission [16]. However, up to 45% of patients may not achieve a serum $fT_{>MIC}$ of 100% when conventional total daily doses (e.g. piperacillin–tazobactam 16–2 g) are administered as a continuous infusion, potentially due to augmented renal clearance [121–123]. The site of infection exposure could be even lower. Currently licensed daily doses of β -lactam antibiotics (piperacillin–tazobactam 16–2 g, ceftazidime 8 g, meropenem 6 g) administered via prolonged or continuous infusions for the treatment of respiratory infections may be insufficient in up to 40% of patients to achieve a $fT_{>MIC}$ of 100% at the site of infection, in part due to highly variable lung tissue penetration (mean/median exposure 20–40% of serum concentrations) [65, 116, 124, 125]. Cefepime achieves approximately equivalent concentrations in serum and the lungs when administered as a continuous infusion, and may be one agent where resistance suppression indices may be met in the lung using continuous infusions of 8 g per day [126]. However, given the wide inter-individual heterogeneity in β -lactam antibiotic pharmacokinetics, TDM may enable individual dose optimisation to facilitate dosing that minimises resistance emergence [122].

5.4 Colistin

Given the use of colistin, administered as the prodrug colistin methanesulfonate (CMS), as a treatment for MDR Gram-negative bacterial infections, the risk of resistance emergence to this agent is likely very high [39]. Plasma concentrations of colistin achieved by commonly used doses may be far below those required for resistance

suppression. For instance, data from a murine thigh and lung infection model suggest that *A. baumannii* resistance to colistin may occur at steady-state concentrations of > 10 mg/l, greatly exceeding the steady-state C_{max} of 2–3 mg/l attainable in critically ill patients by administering 3 million international units (MIU) of CMS thrice daily [45, 127, 128]. However, against *P. aeruginosa*, smaller doses of colistin (~ 3 MIU, resulting in a C_{max} of 3 mg/l) given thrice daily may be adequate to limit the emergence of high-level colistin resistance [128, 129]. Taken together, these data suggest that conventional colistin dosing regimens for clinical efficacy are insufficient to prevent resistance emergence.

Given the pathogen-specific variations in dosing requirements, it may be prudent to consider the highest possible dose that can be safely administered, although what this dose is in different patients is unclear. In a study of 28 critically ill patients, initial loading doses of 9 MIU of CMS followed by twice daily doses of 4.5 MIU did not result in resistance emergence of the initial pathogen, potentially suggesting that the immune response is central to minimisation of resistance emergence [54, 127, 130]. Another approach to achieve high target site exposures for respiratory infections is nebulised dose administration. In one study, nebulised CMS administered at a dose of 1 MIU thrice daily resulted in a median epithelial lining fluid concentration of 6.7 mg/l (interquartile range 4.8–10.1 mg/l) 1 h post-dose, with the respective plasma concentration being fivefold lower [131]. Thus, doses higher than 1 MIU should be administered to achieve adequate concentrations in the lung, whilst limiting systemic exposure. In addition, given the apparent lack of colistin penetration into lung tissue following intravenous CMS administration, concomitant nebulised dosing appears reasonable [132]. The poor lung penetration following intravenous CMS administration would also suggest that intravenous dose optimisation for suppression of resistance against pathogens causing pneumonia is likely to be challenging. At exposures achieved by an intravenous dose, a synergistic combination with other antibiotics may be necessary to suppress emergence of resistance [133]. Alternatively, use of other polymyxins with more predictable pharmacokinetics, like polymyxin B, may prove to be more valuable agents for the treatment of MDR infections. Evidence for this is suggested by a one-compartment dynamic in vitro study that simulated the gradual rise of active colistin concentrations following administration of a loading dose of CMS 12 MIU and 4.5 MIU twice daily thereafter [134]. This was compared with polymyxin B administered as a loading dose of 2 and 1.25 mg/kg daily thereafter against *A. baumannii* [134]. Colistin, administered as CMS, did not result in appreciable bacterial killing, compared with a 6-log reduction in bacteria following polymyxin B

administration; however, this was not sustained, with bacterial regrowth exceeding the baseline within 12 h [134]. No dose of CMS or polymyxin B suppressed resistance emergence [134]. Further increasing the polymyxin B dose has been investigated as one strategy to prevent resistance emergence. In a separate HFIM study, a simulated polymyxin B loading dose of 3.33 mg/kg followed by 1.43 mg/kg twice daily thereafter increased the proportion of the bacterial population able to grow in the presence of 10 mg/l of polymyxin B compared with a loading dose of 2.22 and 1.43 mg/kg thereafter [135]. Indeed, the proportion of the resistant bacterial population was further increased when loading doses of up to 18.3 mg/kg without further maintenance doses were employed [135]. Taken together, these data suggest that polymyxin B should be used in combination with another active antibiotic to minimise resistance emergence.

5.5 Daptomycin

Daptomycin has a spectrum of activity that includes methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) [136].

In one study, the total AUC/MIC ratio of 200 ($fAUC/MIC \sim 20 \text{ h}^{-1}$) [123], achievable with licensed doses of 6 mg/kg/day, was predicted to suppress *S. aureus* daptomycin resistance emergence in a dynamic one-compartment in vitro infection model [137, 138]. However, another in vitro study of *S. aureus* endocarditis vegetations showed threefold increases in daptomycin MICs after use of 6 mg/kg/day, which may be related to reduced daptomycin penetration into the vegetation [139]. Similarly, doses of 6 mg/kg/day have resulted in resistance emergence in a clinical case report [140], potentially due to enhanced daptomycin clearance, which can be seen in critically ill patients [141]. At a simulated dose of 10 mg/kg/day, no decrease in susceptibility to daptomycin was observed in vitro for the *S. aureus* isolates that showed a threefold reduction in susceptibility at a dose of 6 mg/kg/day [139].

5.6 Fluoroquinolones

One-compartment dynamic in vitro studies simulating humanised PK profiles indicate a ciprofloxacin AUC_{0-24}/MIC ratio of $> 285 \text{ h}^{-1}$ or a C_{max}/MIC ratio of > 24 may be required to suppress *P. aeruginosa* resistance [32]. Exposures required for suppression of emergence of resistance are approximately twofold greater than that generally required for clinical cure (AUC_{0-24} of $\geq 125 \text{ mg/l.h}$) in critically ill patients with predominantly respiratory tract infections or Gram-negative bacterial infections [30, 31]. The importance of a C_{max}/MIC ratio for fluoroquinolone efficacy has also been shown in a murine

neutropenic model where a C_{max}/MIC ratio of > 20 correlated with mice survival, potentially by reducing the probability of resistance emergence [142]. However, the AUC/MIC ratio has also been shown to be an important PK/PD ratio for suppression of emergence of resistance. Tam et al. [143], used the dynamic in vitro HFIM and showed that a garenoxacin AUC_{0-24}/MIC ratio of 201 h^{-1} suppressed *P. aeruginosa* resistance emergence. This target may be reduced for *K. pneumoniae* where an AUC_{0-24}/MIC of 67 h^{-1} inhibited resistance emergence, which was also identified in a separate HFIM study [34]. Furthermore, the percentage of the dosing interval spent within the MSW may also be an important PK/PD ratio for emergence of fluoroquinolone resistance. A fluoroquinolone fT_{MSW} of $< 20\%$ is associated with suppression of emergence of resistance [35]. Using this PD target, clinically used intravenous doses of ciprofloxacin 400 mg every 12 h that achieve the clinical target PK/PD exposure for a pathogen with an MIC of 0.125 mg/l is unlikely to prevent suppression of emergence of resistance for isolates with an MIC of $\geq 0.125 \text{ mg/l}$ [66, 144, 145]. Increasing the intravenous ciprofloxacin dose from 400 mg twice daily to 400 mg thrice daily in critically ill patients may be sufficient to suppress the emergence of resistance for isolates with an MIC of $< 0.25 \text{ mg/l}$ [146]. At least one small clinical trial [146] has shown this dosing regimen to be effective and well tolerated.

Other fluoroquinolones with increased Gram-positive antibacterial activity are available, such as levofloxacin and moxifloxacin. It has been shown in dynamic in vitro infection models that fluoroquinolone AUC/MIC ratios between 143 and 431 h^{-1} may suppress MRSA resistance emergence [34, 35]. Simulated clinically used moxifloxacin regimens of 400 mg once daily were sufficient to attain these target PK/PD ratios [35]. In contrast, dose increases of 90, 120 and 540% above currently used clinical doses of 400, 500 and 400 mg twice daily would be required to suppress *S. aureus* resistance emergence for gatifloxacin, levofloxacin and ciprofloxacin, respectively [35]. Lower AUC/MIC ratios of 100 h^{-1} have been shown to minimise resistance emergence of *S. pneumoniae* exposed to moxifloxacin [147]. Importantly, this may be sufficient to minimise the emergence of *S. pneumoniae* resistance in patients without severe sepsis or septic shock, who may require moxifloxacin 400 mg twice daily, depending on the MIC of the pathogen [148, 149].

An important factor regarding fluoroquinolone use is collateral resistance [150]. Commensal organisms, such as faecal *E. coli* and pharyngeal streptococcal species, may become resistant to fluoroquinolones following systemic therapy in up to 33% of patients, possibly related to potential horizontal gene transfer that may propagate the spread of resistance [151–153]. Moreover, the use of

fluoroquinolones increases not only the risk of subsequent fluoroquinolone resistance but also resistance to third-generation cephalosporins, such as ceftriaxone, because of the emergence of extended-spectrum β -lactamase (ESBL)-producing organisms; thus, the judicious use of fluoroquinolones is warranted. Given that further dose increases are unlikely to suppress the emergence of bacterial resistance in the large bacterial inoculum of the faecal flora [152], the fluoroquinolone dose should be targeted to suppress the emergence of antibiotic resistance in the infecting pathogen. Furthermore, consideration should be given to the potential risks of high-dose therapy (e.g. intravenous ciprofloxacin > 400 mg every 8 h), which may include cardiac dysrhythmias and confusion [14].

5.7 Glycopeptides

Vancomycin and teicoplanin are the glycopeptides currently commonly used for MRSA infections. Firsov et al. [38] identified that a vancomycin AUC/MIC > 200 h⁻¹ was sufficient to suppress resistance emergence against MRSA and methicillin-susceptible *S. aureus* (MSSA) in the dynamic one-compartment in vitro infection model, an exposure equivalent to intravenous vancomycin 1 g administered twice daily. These results are consistent with those of Nicasio et al. [154], whereby similar doses against an MRSA isolate resulted in resistance suppression in a dynamic HFIM. These results would suggest that current dosing regimens targeting an AUC/MIC > 400 h⁻¹, which is associated with improved clinical outcomes, is sufficient for suppression of resistance emergence [37]. However, contrasting with these results, Lenhard et al. [39] showed that an AUC/MIC of 1800 h⁻¹ (equivalent to 4 g administered twice daily) was required to suppress vancomycin resistance against two MRSA isolates. The initial inoculum differed between the studies conducted by Lenhard et al. [39] ($\sim 1 \times 10^{10}$ CFU/ml) and Nicasio et al. [154] ($\sim 1 \times 10^6$ CFU/ml), suggesting that the exposure required to suppress resistance emergence depends not only on the specific isolate but also on the bacterial inoculum [155].

Charles et al. [156] described clinical data supporting these in vitro findings; they associated initial trough vancomycin concentrations of < 10 mg/l and high bacterial load infections (infective endocarditis, undrained collections or infected prosthetic material) with emergence of heterogenous vancomycin-intermediate *S. aureus* (hVISA) emergence in MRSA bacteraemia. This is supported by the lack of vancomycin resistance in a patient receiving long-term suppressive vancomycin therapy when trough concentrations were maintained at > 10 mg/l [157].

Taken together, these data suggest high-dose vancomycin therapy is required to mitigate resistance

emergence. A loading dose of 30 mg/kg results in higher trough concentrations than standard doses of 15 mg/kg in the first dosing interval, as shown by Rosini et al. [158], where 34% of patients compared with 3%, respectively, achieved a trough concentration ≥ 15 mg/l. Following a loading dose, a continuous infusion with a steady-state target concentration of 20 mg/l may be sufficient for resistance suppression [159], depending on the pathogen MIC, without an increased risk of nephrotoxicity [160]. Another method to limit glycopeptide resistance emergence may be to reduce the infective inoculum with synergistic combinations. Combinations of vancomycin and ceftazolin [161] or piperacillin–tazobactam [162] or nafcillin [163] have shown rapid reductions and a trend towards shorter clearance times of MRSA and MSSA with high initial inoculums of $\sim 1 \times 10^8$ CFU/ml. The effectiveness of vancomycin and β -lactam combination therapy on clinical outcomes and resistance emergence have yet to be determined in clinical trials [164].

5.8 Linezolid

Little evidence exists regarding PK/PD ratios required for the suppression of resistance emergence. Tsuji et al. [43], in a dynamic HFIM, showed that simulated exposures of 600 mg twice daily dosing (AUC_{0–24} of 124 mg/l.h; AUC/MIC of 112 h⁻¹) suppressed resistance emergence against clinical MRSA isolates, which approximates the AUC/MIC ratio of > 80–120 h⁻¹ associated with improved microbiological eradication [40]. In a separate one-compartment in vitro model, lower linezolid doses of 120 mg twice daily, 120 mg as a continuous infusion over 24 h and 30 mg as a continuous infusion over 24 h were tested against MRSA, hVISA, VISA and VRE isolates [165]. Linezolid resistance emergence, as determined by an increase in MIC, was observed only for the 120 mg continuous infusion regimen, whereby the linezolid concentration approximated the baseline MIC of 2 mg/l for all isolates [165]. This suggests that doses achieving a steady-state linezolid concentration approximately equivalent to the pathogen MIC may promote resistance emergence. In a dynamic one compartment in vitro study investigating vancomycin-resistant *Enterococcus faecalis*, Tsuji et al. [42] showed a variable trend in the linezolid exposure required for resistance suppression. Exposures at an AUC/MIC ratio of > 31 h⁻¹ prevented resistance emergence in all isolates without baseline resistance alleles; however, resistance emergence could not be prevented at any exposure (up to an AUC/MIC of 495 h⁻¹) in a previously susceptible isolate with baseline resistance alleles [42]. Taken together, these data suggest that low linezolid exposures in isolates without baseline resistance mechanisms may result in resistance emergence.

Clinical data regarding linezolid resistance emergence are limited to case reports. In 2010, a linezolid-resistant *S. aureus* outbreak was identified in 12 patients in an ICU in Spain, 11 of whom had previously received linezolid [166]. Similarly, two children receiving suppressive low-dose linezolid therapy were also colonised with linezolid-resistant *S. aureus* [167]. These, in part, may be related to variable linezolid exposures. In critically ill patients receiving linezolid 600 mg twice daily as a 15 to 60 min infusion, only 30% of patients achieved an AUC within the therapeutic range (AUC_{0-24} 200–400 mg/l.h) for a presumed pathogen MIC of 2 mg/l at any point during the study, and only 17% of patients met this target over the 4-day PK study [168]. Of concern is the potential for adverse events, such as haematological toxicity, with a linezolid $C_{min} > 10$ mg/l [169]. Given the variability in linezolid exposures following a standard dosing regimen of 600 mg twice daily and the potential for both poor treatment efficacy and toxicity, TDM has been proposed as one method to improve linezolid dosing practices [170].

6 Impact of the Antimicrobial Regimen and Development of Antimicrobial Resistance

6.1 Combination Therapy

The role of combination therapy has been disputed. Several aspects of patient management intuitively support the use of combination therapy, including extending the spectrum of activity and synergism and minimising resistance emergence. These concepts may not be mutually exclusive. Mathematical models describe the amplification of resistance when a baseline resistant subpopulation is present, which may be present in a seemingly susceptible isolate based on the MIC [50, 171]. Thus, combination therapy may extend the spectrum of activity to the bacterial subpopulation, which may have resistance to one of the antibiotics in the combination regimen [172, 173]. Furthermore, the probability of an organism acquiring a mutation conferring resistance to both antibiotics is significantly less than with monotherapy [74, 174, 175], although developing resistance to two antibiotics would be highly problematic for drug choices. Other potentially beneficial mechanisms relate to the specific antibiotic combination used.

β -Lactams and aminoglycosides often have high rates of synergistic activity against Gram-negative and Gram-positive bacterial pathogens [172, 173, 176]. However, synergistic activity has not been correlated with the suppression of emergence of β -lactam antibiotic resistance against *P. aeruginosa* in vivo [177]. These results are consistent with a meta-analysis that did not identify a

reduced risk of resistance emergence with β -lactam and aminoglycoside combination therapy [178]. In vitro evidence contrasts with clinical trials, whereby low doses of tobramycin and cefepime (3 mg/kg and 563 mg given every 8 h, respectively) suppressed resistance emergence of *P. aeruginosa* in a dynamic HFIM [87]. This discrepancy between clinical studies and in vitro methods may be related to the different dosing regimens used between the meta-analysis, which primarily included studies using multiple daily dosing regimens that may not meet key PK/PD ratios, and the once daily dosing regimens used by in vitro studies. Furthermore, the importance of combination therapy, as demonstrated in vitro studies, may not extend to clinical practice because of the immune system response, the ideal growth conditions provided in vitro that may enhance bacterial growth and the limited bacterial isolates studied in vitro [26]. Further work is needed to characterise the importance of β -lactam antibiotic and aminoglycoside combination therapy to minimise the emergence of resistance [179, 180].

Despite potential β -lactam antibiotic and fluoroquinolone synergistic activity [181, 182], there is an increased risk of emergence of ESBL-producing *Enterobacteriaceae* following fluoroquinolone therapy, which is not seen with aminoglycoside administration, potentially due to mutagenesis mediated by fluoroquinolone exposure [183–185]. Following treatment with a β -lactam antibiotic and fluoroquinolone, 15% of patients subsequently acquired an MDR pathogen compared with 4% of patients who received a β -lactam and macrolide combination in the management of community-acquired pneumonia [186]. This collateral resistance suggests fluoroquinolone use should be restricted to patients unable to tolerate β -lactams and/or aminoglycosides.

Given the increasing emergence of resistant Gram-negative bacteria, fosfomycin has been revived for clinical use; however, concerns exist regarding resistance emergence with monotherapy, and therefore combination regimens have been tested [100]. In an HFIM study, simulating airway exposure from nebulised doses, fosfomycin and amikacin combination therapy reduced the resistance emergence against *P. aeruginosa* that was observed with fosfomycin monotherapy [175]. In another in vitro study, meropenem and fosfomycin combinations following simulated intravenous dosing resulted in bacterial eradication and associated lack of resistance emergence [47]. Clinical studies of fosfomycin contrast with in vitro results, whereby limited resistance emergence has been identified with monotherapy [100]. Until further evidence is available, it may be prudent to use fosfomycin in combination with high-dose aminoglycosides or a carbapenem to prevent resistance emergence.

Given concerns regarding colistin hetero-resistance associated with monotherapy, use of colistin in combination therapy with another agent may reduce the emergence of colistin resistance [187, 188]. Combination therapy with a carbapenem, even if the isolate is resistant to the carbapenem, may prevent colistin resistance emergence [189, 190] and may lead to reduced mortality rates in MDR *A. baumannii* or *P. aeruginosa* infections [191]. Colistin in combination with tigecycline prevented tigecycline resistance emergence but not colistin resistance in vitro with simulated serum concentrations of both agents [192]. Polymyxin B has also been used in combination studies against MDR pathogens. Polymyxin B in combination with fosfomycin in an HFIM suppressed the emergence of two carbapenemase-producing *K. pneumoniae* isolates when a loading dose of polymyxin B 2.5 mg/kg was administered and followed by 1.5 mg/kg twice daily and fosfomycin 6 g as a 1 h infusion four times daily [193]. Interestingly, the same fosfomycin dose administered as a 3 h infusion did not prevent resistance emergence, evident by an MIC increase of sixfold for fosfomycin and 128-fold for polymyxin B [193]. This discrepancy in effect based on the infusion duration may suggest a concentration effect, whereby high peak fosfomycin concentrations are important to have an effect against less-susceptible bacterial subpopulations. Fosfomycin and polymyxin B combination therapy results may not extend to *P. aeruginosa*, with time-kill studies at clinically achievable concentrations resulting in bacterial regrowth [194]. Polymyxin B has also been used in combination with carbapenems. An HFIM study investigating a combination of polymyxin B and doripenem showed that a front-loaded continuous infusion polymyxin B regimen achieving a steady-state concentration of 5 mg/l in combination with doripenem suppressed the emergence of polymyxin B resistance over a 10-day course against a dense 1×10^9 CFU/ml starting inoculum of two polymyxin B heteroresistant strains of *A. baumannii* [195]. In contrast to these results, tigecycline in combination with polymyxin B has not shown consistent suppression of emergence of resistance against *A. baumannii* at clinically achievable concentrations in time-kill in vitro experiments [196]. Taken together, these results suggest that polymyxin B or colistin should be used in combination with a carbapenem, even if the identified bacteria are resistant to the carbapenem. Fosfomycin may suppress resistance emergence against *Enterobacteriaceae* but may not have consistent benefit against *P. aeruginosa*.

Little evidence exists regarding combination therapy against Gram-positive bacterial infections with regards to suppression of emergence of resistance. Some data exists with daptomycin and β -lactam antibiotic combinations. In vitro data suggest that daptomycin and low ($0.25 \times$ MIC) concentrations of ampicillin may prolong

the time to emergence of daptomycin resistance in MRSA and VRE isolates [197].

6.2 Emergence of Resistance is Correlated with Duration of Treatment

Current evidence suggests that antimicrobial resistance emergence correlates with the duration of treatment.

Children with respiratory tract infections receiving high-dose (90 vs. 40 mg/kg/day, both in two divided doses) and short-duration (5 vs. 10 days) amoxicillin therapy have reduced subsequent carriage (24 vs. 32% for high-dose and low-dose course, respectively) 28 days after treatment of penicillin-resistant *S. pneumoniae* isolates [198]. Guillemot et al. [199] and Ruhe and Hasbun [200] found similar effects, where previous use of an anti-streptococcal antibiotic at low doses for prolonged durations resulted in subsequent resistance emergence for colonising and bacteraemia *S. pneumoniae* isolates, respectively.

Similar trends appear evident for Gram-negative bacterial infections. For susceptible *E. coli* isolates causing UTIs, a treatment duration of > 7 days was associated with resistance emergence for amoxicillin/ampicillin [odds ratio (OR) 1.79; 95% confidence interval (CI) 1.24–2.58] and trimethoprim (OR 4.62; 95% CI 2.73–7.82) [201]. This effect has also been seen in the treatment of VAP. In patients receiving 8 versus 15 days of treatment, subsequent infection recurrence was less likely to be due to an MDR bacteria (42.1 vs. 62.3%; $p = 0.04$) [202]. For patients requiring ICU admission with *P. aeruginosa* infections, a duration of therapy > 15 days of meropenem (OR 10; 95% CI 19.8–551), piperacillin–tazobactam (OR 4.7; 95% CI 1.8–12.4), ciprofloxacin (OR 14.5; 95% CI 2.8–75) or ceftazidime (OR 2.6; 95% CI 1.1–6) was associated with resistance emergence, but not for amikacin [203]. However, unlike the other agents investigated, meropenem resistance emergence may occur within 8 days of treatment initiation (OR 6.3; 95% CI 2.4–16.8). Importantly, resistance emergence following therapy may not occur with therapy durations < 4 days [203]. These data suggest that therapy should involve a high dose with the shortest possible duration of therapy, particularly with the carbapenems. Evidence for the treatment of intra-abdominal sepsis is similar to findings stated previously. Sawyer et al. [204] did not find a reduction in the rate of resistant organisms identified in subsequent infections, intra-abdominal or otherwise, between patients receiving 4 ± 1 or up to 10 days of antibiotic therapy. In contrast, patients with anastomotic leakage after colorectal cancer surgery who received > 5 days of antibiotics before the leakage event were twice as likely (OR 2.48; 95% CI 1.18–5.2) to acquire an MDR pathogen in either blood or

peritoneal fluid, suggesting the importance of the inoculum effect for resistance emergence [205].

6.3 Biomarkers for Reducing Antibiotic Exposure

Biomarkers may assist in determining whether antibiotic therapy is indicated and in guiding the duration of antibiotic treatment.

An important distinction that may reduce the use of antibiotics is between sepsis, defined as a systemic inflammatory response syndrome (SIRS) with a presumed or documented infection, and SIRS secondary to a non-infectious cause. This can be difficult based on clinical observations alone. Many biomarkers have been reported in both animal and human trials to diagnose sepsis, but only procalcitonin and C-reactive protein (CRP) are currently available for widespread clinical use, albeit with potentially limited diagnostic value [206–208].

A meta-analysis conducted by Wacker et al. [209] showed that procalcitonin is sufficiently sensitive and specific to distinguish between infectious and non-infectious SIRS in a heterogeneous patient population in the emergency department and the ICU. Significant heterogeneity existed between studies, potentially reflecting differences in patient populations and procalcitonin cut-off values for infection diagnosis. Uzzan et al. [210] identified similar results in post-operative or trauma patients to distinguish SIRS from infection, evident with an area under the receiver operator curve of 0.85. In contrast, in a separate systematic review and meta-analysis [211], procalcitonin was unable to distinguish sepsis from SIRS with an area under the receiver operator curve of 0.79. Although these meta-analyses appear similar, differences in the included articles and the different procalcitonin cut-off points within the included articles in the respective meta-analyses likely limit a direct comparison of the area under the receiver operator curve values. Like any diagnostic test, the clinician must integrate the pre-test probability, involving the background prevalence and the clinical presentation of the patient when deciding on the diagnosis and treatment course.

These decisions regarding treatment initiation are often encountered in the emergency department. van der Does et al. [212] conducted a systemic review and observed that procalcitonin-guided decision making reduced antibiotic use in adults with respiratory complaints but not in paediatric patients. The impact of procalcitonin-guided antibiotic initiation is likely underestimated given the high rates of physician non-adherence in the included studies, which ranged from 6 to 20% [212]. As antibiotic use is clearly correlated with resistance emergence, at both the individual patient [213] and the national level [214], the

use of biomarkers to guide antibiotic commencement would be valuable.

The use of procalcitonin-based clinical algorithms has been shown to reduce antibiotic duration in patients with sepsis or septic shock of varying aetiologies without increasing mortality [215–219]. Antibiotic duration reductions with use of procalcitonin vary but can be reduced by approximately 1 to 4 days. Despite reductions in antibiotic durations, the effect on resistance emergence remains largely unknown. The PRORATA trial is the only procalcitonin trial thus far to investigate the rates of emerging MDR bacteria taken from routine microbiological specimens. Despite a 23% relative reduction in days of antibiotic exposure in the procalcitonin group, no difference in the detection of MDR bacteria was observed [10]. It may be that either this reduction in duration of antibiotic therapy is insufficient to minimise resistance emergence or a more sensitive study design is required. Few other sepsis biomarkers are currently available for routine clinical use.

CRP is another currently available, clinically validated biomarker that may be useful in guiding antibiotic therapy [220]. The sensitivity (75 vs. 88%) and specificity (67 vs. 81%) of CRP is lower than procalcitonin in the diagnosis of bacterial infections; however, trends over time may provide information regarding infection resolution and prognosis [221, 222]. Despite being less specific and sensitive for diagnosing bacterial infections, no difference in antibiotic discontinuation rates in patients with severe sepsis have been identified between CRP and procalcitonin [223]. No studies have yet been conducted examining a reduction in antibiotic use based on CRP concentrations.

Further research is required to identify any reduction in resistance emergence with shorter antibiotic durations, particularly for resistance mechanism carriage in the gastrointestinal tract and in infection recurrence with resistant pathogens.

7 Conclusion

Current antibiotic dosing regimens that are presumably sufficient for clinical resolution of infections are potentially insufficient to prevent resistance emergence. However, current PK/PD ratios that predict resistance suppression are largely derived from *in vitro* studies that do not consider immune system involvement. Future studies are required to define antibiotic exposures required for suppression of emergence of resistance *in vivo*. For a few relatively well-studied antibiotics, the *in vitro* PK/PD ratios required for suppression of emergence of resistance are consistently high, necessitating doses that may be difficult to achieve with conventional dosing regimens, which may result in an unacceptable risk of toxicity. TDM-guided antibiotic dose

individualisation is one strategy to attain these PK/PD ratios, minimise bacterial multi-drug resistance and minimise potential antibiotic toxicity. Alternative dosing strategies, such as β -lactam antibiotic continuous infusions, should be considered to minimise total daily doses required for attaining the higher PK/PD ratios. When possible, target site administration, such as nebulised aminoglycoside therapy, is useful to achieve high exposures with minimal risk of systemic toxicity. The use of combination regimens to prevent resistance emergence is supported by in vitro experiments but not yet by clinical evidence. Biomarkers such as procalcitonin and CRP are helpful to guide individualisation of the duration of antibiotic therapy, although evidence for minimising resistance is lacking. Overall, current knowledge on antibiotic strategies that prevent resistance emergence is meagre. Future studies should describe appropriate PK/PD predictors or clinical biomarkers associated with resistance suppression and validate their utility for individualising therapy at the bedside.

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Compliance with Ethical Standards

Conflict of interest Jason Roberts would like to declare collaborations with MSD, Bayer, Astellas, Pfizer, bioMerieux and Accelerate Diagnostics but none of which are considered relevant to the content of this paper. Aaron Heffernan, Fekade Sime and Jeffrey Lipman have no conflicts of interest to declare.

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