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Optimising Dosing Strategies of Antibacterials Utilising Pharmacodynamic Principles

Impact on the Development of Resistance

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Abstract

Evolving antimicrobial resistance is of global concern. The impact of decreased susceptibility to current antibacterials coupled with the decline in the marketing of new agents with novel mechanisms of action places a tremendous burden on clinicians to appropriately use available agents. Optimising antibacterial dose administration through the use of pharmacodynamic principles can aid clinicians in accomplishing this task more effectively. Methods to achieve this include: continuous or prolonged infusion, or the use of smaller doses administered more frequently for the time-dependent β-lactam agents; or higher, less frequent dose administration of the concentration-dependent aminoglycosides and fluoroquinolones. Pharmacodynamic breakpoints, which are predictive of clinical and/or microbiological success in the treatment of infection, have been determined for many classes of antibacterials, including the fluoroquinolones, aminoglycosides and β-lactams. Although surpassing these values may predict efficacy, it may not prevent the development of resistance. Recent studies seek to determine the pharmacodynamic breakpoints that prevent the development of resistance. Numerous studies to this point have determined these values in fluoroquinolones in both Gram-positive and Gram-negative bacteria. However, among the other antibacterial classes, there is a lack of sufficient data. Additionally, a new term, the mutant prevention concentration, has been based on the concentrations above which resistance is unlikely to occur. Future work is needed to fully characterise these target concentrations that prevent resistance.

Evolving antimicrobial resistance is of global concern. Resistance rates are increasing among Gram-positive and Gram-negative organisms that threaten the clinical efficacy of antibacterial therapy both in the inpatient and outpatient setting.[1] Equally as concerning is the fact that very few new antibacterials are in the pipeline to be marketed.[2] The ideal situation would be if new antibacterials with novel mechanisms of action soon became available because there would be a very high probability that multidrug-resistant bacteria would be susceptible to these antibacterials. Such examples that have recently been marketed include linezolid, an oxazolidinone, and daptomycin, a cyclic lipopeptide, which are principally used clinically to treat vancomycin-resistant enterococci (VRE) and

methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, telithromycin, a ketolide, has been developed to target *Streptococcus pneumoniae*. Among these new antibacterials, tigecycline, an agent structurally similar to the tetracyclines, is the only agent with activity against Gram-negative pathogens, with the exception of *Pseudomonas aeruginosa*, and no new agents are scheduled to be marketed soon. Because of this, older, more toxic agents, such as the polymixins, are regaining use.

Because of the lack of new agents, it is imperative to properly use currently available antimicrobials. In addition to proper antimicrobial stewardship, one way to ensure this is by determining the most effective dosing regimen that maximises the probability of bacterial eradication. The field of pharmacodynamics attempts to determine these relationships. Pharmacodynamics is a discipline that relates the antimicrobial concentration achieved in the body to a measure of bactericidal activity. In other words, it attempts to describe the interaction between drug and bug.[3] Throughout this review, data are presented detailing the pharmacodynamic relationships and breakpoints that predict clinical and/or microbiological efficacy for certain classes of antibacterials, specifically fluoroquinolones, β-lactams and aminoglycosides. Although these data have been extensively evaluated, very little information exists describing the pharmacodynamic targets that need to be achieved with clinical dose administration to prevent the development of resistance. Available data are presented and its implications on current dosing strategies are discussed.

1. Antibacterial Resistance

Resistance among the most commonly encountered Gram-positive pathogens, *S. aureus*, *S. pneumoniae* and enterococci have dramatically increased over the past two decades. As reported from the SENTRY Antimicrobial Surveillance programme, MRSA was the most frequent pathogen demonstrating a high degree of nosocomial transmission in countries throughout the world.[4] In the US, the National Nosocomial Infection Surveillance (NNIS) service has noted marked increases in resistance rates among *S. aureus* (from 28% in 1989 to 57.1% in 2002).[5] The emergence of penicillin-resistant *S. pneumoniae* (PRSP) has also caused concern specifically regarding the use of β-lactam antibacterials to treat lower respiratory tract infections both in the US and in other countries including Canada, Northern Ireland and Spain.[6] Current resistance rates for β-lactams obtained during the respiratory season of 2001–2 have increased to 18.4% for penicillin and 1.7% for ceftriaxone, as reported by the TRUST (Tracking Resistance in the United States Today) surveillance programme.[6] The same concerns apply to macrolides, the alternative class of antibacterials used as monotherapy for lower respiratory tract infections in the outpatient setting, with *S. pneumoniae* resistance >25% as well as increasing minimum inhibitory concentration (MIC) values within the susceptible range.[6] Fluoroquinolone resistance in the US is generally considered low (<2%), but isolated reports of potentially significant resistance rates to ciprofloxacin and levofloxacin have been reported in other countries such as Canada[7] and Hong Kong.[8] Furthermore, these strains are also present in the US and result in clinical failure.[9,10] For enterococci, the latest report from the NNIS shows that the VRE rate among nosocomial infections in patients admitted to the intensive care unit (ICU) was 27.5% in the year 2002, an 11% increase compared with the average resistance rate of the previous 5 years (1997–2001).[5]

The susceptibility rates among antibacterial agents targeting Gram-negative pathogens continue to decrease as well. Of particular concern are multidrug-resistant *P. aeruginosa*, extended-spectrum β-lactamase (ESBL)-producing isolates of *Escherichia coli* and *Klebsiella* spp., and stably derepressed *AmpC* β-lactamases (principally in *Enterobacter* spp.). Among bloodstream isolates in the year 2002, the rates of multidrug-resistant *P. aeruginosa* were much higher in Latin America (18.7%) and Europe (11.5%) than in North America (3.0%).[11] However, rates of multidrug resistance (defined as resistance to three or more drugs) among

isolates obtained from the ICUs in the US has increased from 4% in 1993 to 14% in 2002.[12] Another study has reported multidrug-resistant *P. aeruginosa* in the US to have increased from 12.8% in 1997 to 20.8% in the year 2000.[13] Although the percentages cited in various studies may be different as a result of factors such as the location of the patient when cultured (i.e. ICU or non-ICU), the site the isolate was located (bloodstream vs respiratory tract vs all sites), or from the definition of multidrug resistance, all of these studies do show that multidrug-resistant *P. aeruginosa* is increasing. Enterobacteriaceae containing an ESBL phenotype have been noted in many areas of the world including the US, the Western Pacific, Europe and Canada, and the prevalence has been reported as high as 45% in Latin America. These resistance mechanisms can be in part due to increased utilisation of certain antibacterial agents, as has been demonstrated with the correlation of increased ciprofloxacin use coupled with a severe decrease in susceptibility,[14] or can be induced during antibacterial treatment as a result of the overexpression of a β-lactamase, for instance *AmpC* among *Enterobacter* spp. secondary to cephalosporin use, or some combination of these factors.[15]

2. Antibacterial Pharmacodynamics

Maxwell Finland once said that, "We know everything else about antibiotics but how much to give".[16] As a science, antibacterial pharmacodynamics describes the relationship between the drug concentration and the antibacterial effect, as referenced to the MIC. Through the use of pharmacodynamic principles, optimal dosing regimens can be determined that will maximise antibacterial activity.

In general, antibacterial agents are classified into two classes on the basis of their antibacterial killing patterns. These include concentration- and time-dependent killing, which are further described in sections 2.1 and 2.2. Additionally, it is important to note several concepts that should be applicable upfront to all pharmacodynamic analyses. First, it is ideal to measure and report the free drug concentration in pharmacodynamic experiments, as this is what is available to bind to bacteria and exhibit an antibacterial effect. Secondly, the free drug has to be in proximity to bacteria (i.e. at the site of infection). Because of the practical limitations with obtaining fluid or tissue specifically at the infection site, however, the blood serum concentration is typically used as it is more easily obtained. Throughout this review, unless otherwise specified, the antibacterial concentrations used to determine pharmacodynamic parameters have been obtained from serum.

2.1 Concentration-Dependent Killing

Concentration-dependent antibacterials display an increased rate and extent of bacterial kill with increasing drug concentrations. Therefore, increasing the peak (maximum) concentration (C_{max}) above the minimum concentration necessary to inhibit growth of the organism (C_{max}/MIC) maximises bacterial eradication (figure 1). Aminoglycosides, fluoroquinolones and metronidazole are examples of antibacterials that demonstrate concentration-dependent antimicrobial activity at their standard dosages (table I).

Aminoglycosides display concentration-dependent bactericidal activity and outcomes data from human studies have demonstrated the importance of a high free (*f*) C_{max}/MIC. Deziel-Evans et al.[18]

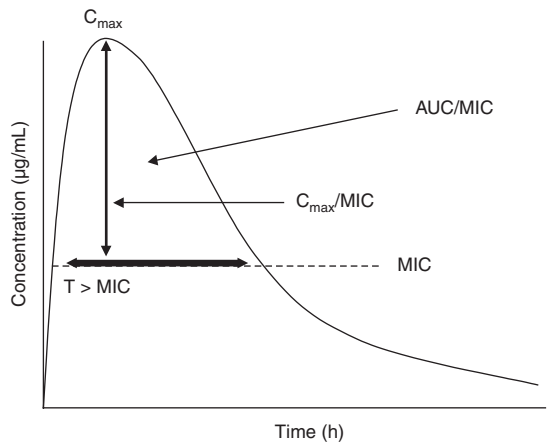

Fig. 1. Pharmacodynamic indices that describe antibacterial killing. **AUC** = area under the concentration-time curve; **C_{max}** = maximum (peak) concentration; **MIC** = minimum inhibitory concentration; **T** = time.

Table I. Pharmacodynamic parameters that correlate with efficacy[17]

	C_{max}/MIC	AUC/MIC	T > MIC
Examples	Aminoglycosides Fluoroquinolones	Azithromycin Fluoroquinolones Telithromycin	β-Lactams (penicillins, cephalosporins, carbapenems) Macrolides (clarithromycin, erythromycin) Oxazolidinones (linezolid)
Organism kill	Concentration dependent	Concentration dependent	Time dependent
Therapeutic goal	Maximise exposure	Maximise exposure	Optimise duration of exposure

AUC = area under concentration-time curve; **C_{max}** = maximum (peak) concentration; **MIC** = minimum inhibitory concentration; **T** = time.

demonstrated in a retrospective study that 91% of patients with an *f*C_{max}/MIC >8 achieved clinical cure compared with only 12.5% of patients with an *f*C_{max}/MIC ≤4. Kashuba et al.[19,20] showed that achieving an aminoglycoside *f*C_{max}/MIC ratio of ≥10 within 48 hours of initiation of therapy for Gram-negative pneumonia resulted in a 90% probability of therapeutic response by day 7. To maximise the *f*C_{max}/MIC, extended-interval high-dose regimens of either gentamicin or tobramycin are widely administered in the US. An extended-interval high-dose aminoglycoside regimen has been shown to have equivalent or superior activity with no difference in renal toxicity compared with traditional every-8-hour administration.[21-25]

Fluoroquinolones have also demonstrated improved outcomes at the *f*C_{max}/MIC of 10–12.[26] Because the *f*C_{max}/MIC of 10 cannot be achieved clinically against current bacteria with elevated MICs without dose-related toxicity concerns, the area under the concentration-time curve (AUC) to MIC ratio (AUC/MIC) has been supported by the literature to be the pharmacodynamic parameter that best predicts efficacy for this class. The actual AUC/MIC breakpoint that predicts efficacy for this class of antibacterials has been extensively evaluated throughout the past decade. Forrest and colleagues[27] retrospectively evaluated the relationship of ciprofloxacin exposure with clinical and microbiological response, as well as time to eradication, in 74 patients with serious nosocomial infections. These investigators observed that a total drug AUC/MIC of 125 was a critical breakpoint predicting successful clinical and microbiological outcomes. At an AUC/MIC <125, the percentage probabilities of clinical and microbiological responses were 42% and 26%, and for an AUC/MIC >125, the rates were statistically greater at 80% and 82%, respectively. In addition, two breakpoints were determined to predict time to bacterial eradication. At an AUC/MIC <125, the time to bacterial eradication exceeded 32 days; however, an AUC/MIC >125 and >250 led to a significantly decreased time to eradication, (125–250: 6.6 days, >250: 1.9 days; $p < 0.005$). It is important to acknowledge that the majority of patients included were infected with Gram-negative bacteria and that the authors did not consider the protein binding of ciprofloxacin, which is approximately 20–40%, in their analysis. In another study involving 47 patients with nosocomial pneumonia, a levofloxacin total drug AUC/MIC exposure ≥87 was prospectively determined to be four times more likely to achieve bacterial eradication.[28] This study included both Gram-positive (mainly *S. aureus*) and Gram-negative pathogens, as well as the use of combination therapy with ceftazidime or piperacillin/tazobactam for *P. aeruginosa*. These variables, along with slightly different methodologies, including lack of consideration of protein binding, may partially explain why the pharmacodynamic breakpoints among these studies do not agree.

Most of the studies discussed previously have examined target breakpoints in Gram-negative bacteria. However, it is has been observed that much lower *f*AUC/MIC breakpoints of 30–40 are necessary to achieve positive clinical and microbiological outcomes when targeting Gram-positive organisms such as *S. pneumoniae*.[29] One study, performed by Ambrose and colleagues[29] found a relationship between fluoroquinolone (gatifloxacin and levofloxacin) AUC/MIC and microbiological response. At *f*AUC/MIC exposures ≥33.7, microbiological re-

sponse was 100% compared with 64% at exposures that failed to reach this critical breakpoint. It is important to note that this study evaluated efficacy in the context of wild-type *S. pneumoniae* that were not known to harbor a first-step *parC* gene. From a clinical perspective, growing concern has been expressed that the current *in vitro* susceptibility studies are unable to distinguish *S. pneumoniae* with first-step mutations.[10,30] Numerous studies have demonstrated a much greater likelihood for pneumococci to acquire a second-step mutation if the first-step mutant is already present.[31,32] This second-step mutation leads to a dramatic increase in MIC, in which adequate exposures necessary for efficacy may no longer be obtained.

2.2 Time-Dependent Killing

Time-dependent killing agents exhibit bactericidal activity when the free concentration of drug remains above the MIC (time above MIC, *f*T > MIC) of the pathogen for a certain time (figure 1). Maximal efficacy is observed up to only two to four times the MIC of the pathogen.[17] β-Lactams (e.g. penicillins, cephalosporins, monobactams, carbapenems), lincosamides (e.g. clindamycin), macrolides (e.g. erythromycin, clarithromycin), glycopeptides (e.g. vancomycin) and oxazolidinones (e.g. linezolid) demonstrate this bactericidal pattern (table I).

β-Lactams require varying *f*T > MIC according to the results of *in vitro* and *in vivo* animal studies. For instance, carbapenems require 20% and 40% of *f*T > MIC for bacteriostatic and bactericidal activity, whereas cephalosporins require 35–40% for bacteriostasis and 60–70% for maximum bactericidal activity.[3,33,34] Penicillins generally inhibit bacterial growth at 30% *f*T > MIC and achieve bactericidal exposures at 50% *f*T > MIC.[3]

A review by Turnidge[34] discusses data from *in vitro*, *in vivo* and clinical studies that have provided the rationale for β-lactam time-dependent bactericidal activity. However, data have only recently been presented specifically evaluating if failure to achieve pharmacodynamic targets predict efficacy. Only one study included in the review by Turnidge[34] addressed this issue. Schentag and colleagues[35] examined the efficacy of cefmenoxime in critical care patients with nosocomial pneumonia. These investigators concluded that the AUC above the dynamic response concentration, an alternative to the MIC that represents the time to eradication of the pathogen from tracheal secretions, was the strongest predictor of clinical efficacy. The review by Turnidge points out a limitation to the study and upon re-analysis observes that the T > MIC was the strongest predictor ($r^2 = 0.815$). In a separate review in patients with otitis media and acute maxillary sinusitis, it appears that a T > MIC of approximately 40% for penicillins and 50% for cephalosporins achieves high bacteriological eradication rates.[36] More recently, the pharmacodynamics of cefepime (plus aminoglycoside) were determined in 20 patients with Gram-negative infections.[37] These investigators observed a strong link between microbiological success and T > MIC. Success was 89% when the total T > MIC was 100% compared with 0% when T > MIC was <100% ($p = 0.032$). Utilising classification and regression tree analysis (CART), a minimum concentration (C_{min})/MIC >4.3 was independently predictive of response. These investigators determined that in order to achieve a probability of 80% and 90% microbiological success, serum concentrations would need to exceed 4.3 × MIC for 83% and 95% of the dose administration interval, respectively. Similarly, Lee and colleagues[38] evaluated clinical cure in patients receiving cefepime monotherapy against ESBL and non-ESBL strains of *E. coli* and *Klebsiella* spp. Eradication was 80% when the total drug T > MIC was 100% compared with 0% when T > MIC was <100% ($p = 0.025$). CART identified that C_{min}/MIC was more predictive of eradication than T > MIC. Regardless of ESBL production, all pathogens were eradicated when C_{min}/MIC >8.9 and only 33.3% were eradicated when C_{min}/MIC ≤8.9 ($p = 0.009$). Although these studies only included a small group of patients, they suggest that higher pharmacodynamic targets than previously documented from *in vitro* and animal studies may be needed in the clinical setting. Future studies will be necessary to

Table II. Pharmacodynamic breakpoints established in the literature[3,17,19,20,27,29,33,34,44-47]

Antibacterial class	Pharmacodynamic parameter that best describes activity	Breakpoints for clinical or microbiological efficacy	Breakpoints for prevention of resistant mutant formation
β-Lactams	*f*T > MIC	Penicillins: 50% Cephalosporins: 50–70% Carbapenems: 40%	Not defined
Aminoglycosides	fC_{max}/MIC	≥10–12	Not defined
Fluoroquinolones	AUC/MIC	Gram-negative bacteria: total AUC/MIC ≥125 Gram-positive bacteria: *f*AUC/MIC ≥30	Garenoxacin: total AUC/MIC ≥190 Levofloxacin: total AUC/MIC ≥157 *Streptococcus pneumoniae*-containing *parC*: total AUC/MIC ≥200

AUC = area under concentration-time curve; $\mathbf{C}_{max}$ = maximum (peak) concentration; ***f*** = free drug concentration; **MIC** = minimum inhibitory concentration; **T** = time.

fully characterise this pharmacodynamic relationship.

Although β-lactams have traditionally been administered by intermittent infusion, continuous or prolonged infusion is gaining attention because of the time-dependent pharmacodynamics of these agents and potential economic savings. While earlier comparison between continuous infusion and intermittent infusion of β-lactams reported similar clinical and microbiological outcomes,[39-41] Krueger et al.[42] through the use of Monte Carlo simulation with MYSTIC surveillance data reported that the continuous infusion of meropenem provided a better likelihood of attaining a bactericidal exposure against *P. aeruginosa* compared with the intermittent administration (83% vs 64%). Tam et al.[43] also found that continuous infusion of cefepime 4g over 24 hours provided a much higher cumulative fraction of response of 65–81% ($p < 0.001$) compared with standard dose administration of 2g every 12 hours (4–38%) against *P. aeruginosa* isolates from their institution.

3. Optimising Pharmacodynamic Principles to Prevent the Emergence of Resistance

Much of the research performed thus far has attempted to determine the pharmacodynamic breakpoint that best describes the bactericidal activity of specific antibacterial agents. However, correlating pharmacodynamic breakpoints with resistance prevention has recently been described, specifically for the fluoroquinolone class (table II).

Within any population of bacteria causing infection there is a range of susceptibilities of the organisms to a specific antibacterial used. Although a small, more resistant subset of organisms is present, it may not constitute the dominant population and it is not able to grow because of the competition for nutrients among a whole population. However, when an antibacterial is introduced, the more susceptible or dominant population is easily eradicated, leaving only the more resistant subpopulation. If an antibacterial is not given in the optimal dosage regimen to ensure eradication of all of the organisms, growth of the more resistant subpopulation will proliferate until this organism now constitutes the majority of organisms.[3] In this situation, the antibacterial regimen has selected out the resistant subpopulation and resistance has developed (figure 2). The goal of antibacterial therapy should consider not only eradicating the dominant pathogen but also achieving an exposure that prevents the growth of the resistant subpopulation, thereby preventing resistance.

Although not discussed further in this review, a similar concept might apply to bacteria in a biofilm (most notably *S. aureus* and *P. aeruginosa*). While debate remains regarding the ability of antibacterials to penetrate biofilms, recent studies document genetic changes in bacteria at the core, which communicate through quorum sensing.[48] Because these bacteria do not require replication for survival in the biofilm environment, they are not affected by

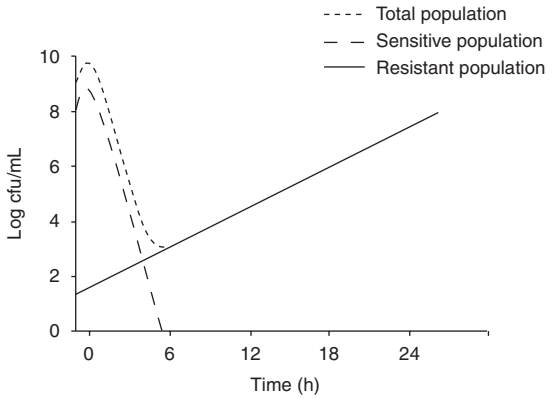

Fig. 2. The emergence of resistance. At time = 0, a bacterial population has a variety of minimum inhibitory concentrations against a specific pathogen. After exposure to the antibacterial, the sensitive population is rapidly eradicated, leaving only the drug-resistant population. Because of the lack of competition for nutrients, the resistant subpopulation is allowed to grow to the level of the original population. Selection for resistance has occurred (reproduced from Drusano,[3] with permission from *Nature Reviews Microbiology* [www.nature.com/reviews], copyright 2004 Macmillan Magazines Ltd).

antibacterials; however, they are simultaneously able to mutate and become resistant over time. Therefore, when they do become planktonic, they are likely to already be drug resistant.

3.1 Fluoroquinolones

The most significant research to date on the use of pharmacodynamics to prevent resistance has been conducted with the fluoroquinolones. Thomas and colleagues[49] retrospectively evaluated 107 patients treated in four separate clinical trials of nosocomial lower respiratory tract infection. These authors identified that a total AUC/MIC ≥100 significantly decreased the probability of developing resistance while receiving therapy. This relationship held among all treatment groups and all pathogens except for β-lactamase-producing Gram-negative organisms. However, these data have been criticised for the time differential (7–10 years) among the patients included in the analysis and for the combination of data from different drug classes that express different pharmacodynamically linked variables (e.g. β-lactams: T > MIC, fluoroquinolones: AUC/MIC and then analyse the data with only AUC/MIC).[33]

Recently, work relating to the pharmacodynamic target needed to prevent the development of resistance has come from Drusano and colleagues.[44,45] The first study utilised a standard mouse-thigh infection model to determine which doses of levofloxacin would amplify or suppress drug-resistant subpopulations of *P. aeruginosa*.[44] From these data, a mathematical model was validated and determined that achieving a total AUC/MIC of 157 predicted a high likelihood of achieving resistance suppression in humans. Through use of a 10 000 subject Monte Carlo simulation, the authors determined that levofloxacin 750mg every 24 hours would reach this target AUC/MIC only 61.2% of the time. The authors also simulated ciprofloxacin 400mg every 8 hours and found a similar cumulative fraction of response of 61.8% against 404 *P. aeruginosa* isolates. The second study evaluated the ability of a newer desquinolone, garenoxacin, to suppress resistant mutant growth against *P. aeruginosa*.[45] Through use of an *in vitro* hollow fibre infection model, these investigators performed several time-kill curves over 48 hours using different exposures (AUC/MICs) of the drug to determine its effect on both the total population and resistant subpopulation. From these data, the authors developed a mathematical model, similar to the one developed in their first study,[44] that predicted the drug exposure necessary to suppress amplification of the resistant subpopulation. The authors then validated the mathematical model by performing another *in vitro* experiment targeting slightly above and below the target value. These investigators determined that achieving a critical total drug AUC/MIC of 190 suppressed amplification of the antibacterial-resistant subpopulation. Resistant bacteria emerged at exposures close to but below this value.

Evidence has been presented questioning if the accepted AUC/MIC breakpoint of 30–40 for fluoroquinolones against *S. pneumoniae* is adequate in isolates containing a pre-existing *parC* mutation. In a study performed by Florea and colleagues,[46] levofloxacin was found to be ineffective against *parC* mutants when simulating epithelial lining fluid concentrations of 500mg every 24 hours in older

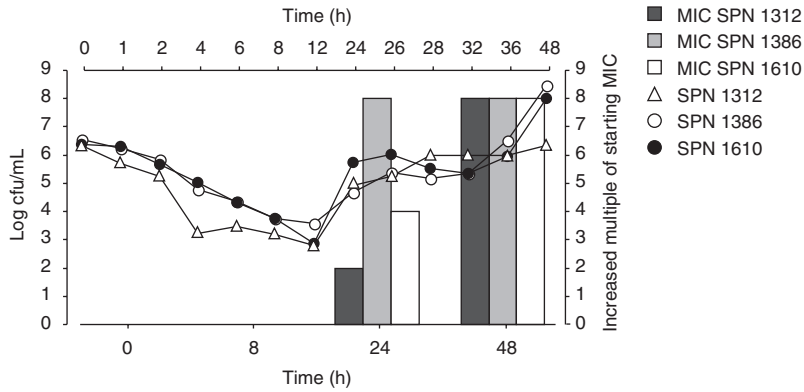

Fig. 3. Antibacterial and resistance profiles of *Streptococcus pneumoniae* (SPN)-containing *parC* ± *parE* mutations after simulated human epithelial lining fluid concentrations of levofloxacin. All three isolates had an initial levofloxacin minimum inhibitory concentration (MIC) of 2 μg/mL (reproduced from Florea et al.,[46] with permission from the American Society for Microbiology).

adults who were undergoing scheduled diagnostic bronchoscopy for probable pulmonary pathology even though the AUC/MICs achieved (100–200) were much higher than the accepted AUC/MIC breakpoint of 30 (figure 3). On the other hand, as a result of the much higher exposures achieved simulating moxifloxacin 400mg every 24 hours of 800–1600, due to much lower MICs, bacterial eradication occurred with a lack of development of resistant mutations. A limitation of this study was that similar exposures were not compared between these two fluoroquinolones. Therefore, in a separate experiment, through the use of an *in vitro* pharmacodynamic model, the target moxifloxacin AUC/MIC was lowered and levofloxacin exposure was raised to examine if differences in bactericidal activity existed between these two agents.[47] At exposures of approximately 200–400, both of these agents achieved bactericidal efficacy and prevented the emergence of resistance (figure 4). Genotypic analysis confirmed that second-step mutations did not develop after the 48-hour time interval when this exposure was achieved.

A concept separate from pharmacodynamics has been described to restrict the development of resistant mutants. The mutant prevention concentration (MPC) describes the antibacterial concentration that inhibits the growth of the least-susceptible, single-step mutant; it is essentially the MIC of the least susceptible organism.[50] There is a low likelihood for spontaneous mutant formation at or above the MPC. This concept is developed further by the description of the mutant selection window (MSW), which describes the concentration range between the MPC and MIC in which resistant mutants are likely to develop. This concept has been primarily applied to the fluoroquinolones because the primary resistance mechanism, spontaneous chromosomal point mutations measured in the laboratory, is the same mechanism of resistance as observed clinically.

A few key points have been learned from data thus far. First, the MPCs differ among the various

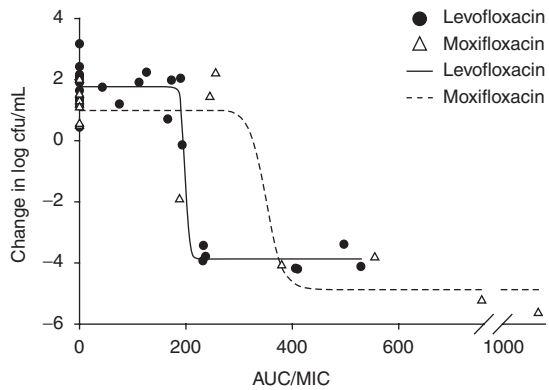

Fig. 4. Bactericidal activity at 48 hours as a function of exposure for moxifloxacin and levofloxacin. It appears that the critical exposure necessary to prevent the development of resistance is a total area under the concentration-time curve (AUC)/minimum inhibitory concentration (MIC) ratio of between 200 and 400.[47]

fluoroquinolones against different pathogens.[51] Among the Gram-positives, the concentration that inhibits emergence of resistant mutants in 90% of the isolates tested (MPC_{90}) against *S. pneumoniae* include: gemifloxacin 0.5 μg/mL, moxifloxacin 1 μg/mL, gatifloxacin 2 μg/mL and levofloxacin 4 μg/mL. Against 100 methicillin-susceptible *S. aureus* strains, the MPC values were moxifloxacin 0.25 μg/mL, gemifloxacin 0.5 μg/mL, gatifloxacin 1 μg/mL and levofloxacin 1 μg/mL. The newer fluoroquinolones, gatifloxacin, moxifloxacin and gemifloxacin, have the ability to bind to both topoisomerase II and IV, instead of just one of these targets, to provide efficacy.[52] This may be the reason that these agents have demonstrated lower MPCs than the older fluoroquinolone levofloxacin. With regard to Gram-negatives, fluoroquinolones have been tested against 100 clinical isolates of *P. aeruginosa*, and the MPC for ciprofloxacin was 2 μg/mL compared with 8 μg/mL for levofloxacin.[53] Secondly, the MPC for each antibacterial agent is dependent on the genotypic profile of the organism. For example, through use of an *in vitro* pharmacodynamic model, Allen and colleagues[31] demonstrated that administration of levofloxacin and moxifloxacin at MPC-targeted regimens prevented the development of resistance in fluoroquinolone-susceptible isolates, with MPC values for moxifloxacin and levofloxacin of 0.5 μg/mL and 2 μg/mL, respectively. However, these regimens were unable to prevent the selection of resistance in mutants already containing *parC,* where the MPC values for moxifloxacin and levofloxacin were 4 μg/mL and 32 μg/mL, respectively. Similarly, Smith and colleagues[32] evaluated the rank order of MPC values among various fluoroquinolones against *S. pneumoniae* isolates with varying genotypic profiles. These investigators determined that once a *parC* mutation was present, the MPC of all fluoroquinolones rose dramatically. Finally, the amount of time the drug concentration remains above the MPC may also be a critical factor predicting efficacy. For example, if the pharmacokinetic profile of each of the fluoroquinolones is taken into account, then the time the serum drug concentration remains above the MPC_{90} against *S. pneumoniae* is >24, >12, >6 and ~3–4 hours for moxifloxacin, gemifloxacin, gatifloxacin and levofloxacin, respectively.

Although a new and potentially useful technique, a few limitations exist that compromise the utility of the method and validity of the results. From a technical standpoint it may be difficult to obtain the desired inoculum of 10^{10} cfu, especially with respect to *S. pneumoniae*, an organism that is susceptible to autolysis. Also, Blondeau et al.[51] determined that using the standard agar dilution method of MPC determination overestimated the true MPC by approximately 2-fold against *S. pneumoniae*. To circumvent this, the authors reported a provisional MPC, which was one 2-fold dilution less than observed in the experiment. Although this may be considered a conservative estimate of the true MPC, it certainly calls into question the validity of the results using this methodology. From an applicability standpoint, all of these data describing the MPC and MSW are derived from *in vitro* analyses and have yet to be validated clinically. Similarly, further research establishing a correlation between the MPC and pharmacodynamic effects of each antibacterial agent are still required.

3.2 β-Lactams

In contrast to the fluoroquinolones, sparse data are available examining the exposure necessary for β-lactam agents to prevent resistance. Data have been reported regarding the carriage of PRSP and suboptimal dosing of antibacterials. In a study by Guillemot and colleagues,[54] while describing 16 children carrying PRSP, the authors found an odds ratio (OR) of 5.9 (95% CI 2.1, 16.7; $p = 0.002$) for carrying the resistant isolate if the β-lactam dosage was below the clinically recommended dosage. Although this study did not evaluate the exact T > MIC obtained in these children it did lay the groundwork for future studies. Similarly, in an analysis of different starting concentrations of benzylpenicillin targeted against a mixed population of *S. pneumoniae* with different MICs to penicillin, Odenholt and colleagues[55] determined that the susceptible population was easily eradicated while the resistant subpopulation was, therefore, allowed to

proliferate. Although, again, the exact T > MIC needed to suppress mutant growth was not specifically evaluated, no regrowth was noted when the T > MIC was 48%, 38% and 46% for the penicillin-susceptible, intermediate and resistant subpopulations, respectively. Additionally, this same group tested the effect of a selection of resistant *S. pneumoniae* through the use of insufficient benzylpenicillin treatments with the same bacterial strain in three different animal infection models.[56] With the use of regimens that did not provide a T > MIC of 40–50% of the dose administration interval, selection of resistant pneumococci was observed in the rabbit tissue cage model, the ratio of resistant-to-susceptible bacteria increased in the favour of the resistant population in the mouse thigh infection model, and the susceptible strain was completely overgrown by the less susceptible strains in the mouse peritonitis model. Together, these data all demonstrate the importance of eradication of all bacteria in order to avoid the selection of resistant clones.

Two abstracts by Ong and colleagues[57,58] specifically examined the T > MIC breakpoint that prevents the development of resistance among different β-lactam agents. In the first study,[57] a mouse thigh infection model was used to determine the specific T > MIC that prevented resistance development among *P. aeruginosa* isolates. The bactericidal activity of meropenem was maximal at 30% T > MIC and little resistance developed even when dosing was suboptimal. Because meropenem achieves concentrations in humans that are much higher than 30% T > MIC for susceptible *P. aeruginosa*, selection of resistance is suspected to be rare. In the second analysis,[58] a mouse thigh infection model was used to test for efficacy and the ability to suppress resistant mutant formation of various exposures of meropenem, imipenem and cefepime against a MexA-MexB-OprM efflux mutant of *P. aeruginosa*. Maximal efficacy was noted at 40% T > MIC for each carbapenem and 70% for cefepime. Resistance did not emerge at any T > MIC exposure.

Although these previous studies could not find differentiation between the T > MIC needed to ensure bactericidal activity and suppression of resistant mutants, a recent study demonstrates that selective enrichment of resistant mutants can occur with β-lactams as the susceptible population is eradicated. Tam and colleagues[59] examined the propensity of different β-lactam agents (piperacillin, ceftazidime and meropenem) to suppress spontaneous resistance among a dense population, approximately 10^8 cfu/mL, of *P. aeruginosa*. Time-kill studies were performed at six clinically achievable constant concentrations of each agent in relation to the MIC of the organism (i.e. 0.25 × MIC, 1 × MIC, 4 × MIC). A resistant subpopulation was evident after exposure to all three agents. The amount of regrowth of the resistant subpopulation was due to the bactericidal activity of each agent against the dominant, more susceptible bacterial population. If more bacteria were eradicated, a selective advantage existed for the resistant subpopulation and they were able to grow. Piperacillin had only marginal activity against this pathogen, most probably as a result of the inoculum effect, yet did not yield many resistant organisms. Meropenem, on the other hand, which was least affected by the inoculum effect, led to a ≥2 log reduction in organisms at a concentration of 4 × MIC but yielded growth of resistant subpopulations to the greatest extent. Suppression of the resistant clones was only observed once the meropenem concentrations reached 16 × MIC. These investigators determined that different β-lactam subclasses have a distinct killing profile and propensity to select resistance. An important consideration is that this study did not correlate human exposures to the ability to suppress growth of resistant subpopulations and this will be an area in which research is needed in the future.

Another method commonly cited to suppress the emergence of resistance with the β-lactams is the use of combination therapy. Currently, the most widely accepted combination antimicrobial therapy involves the addition of an aminoglycoside to a β-lactam against *P. aeruginosa*. However, from a clinical perspective, considerable debate continues

as to whether combination therapy reduces the emergence of resistance in *P. aeruginosa*.[60] In recent studies, no difference with respect to emergence of resistance has been shown between mono- and combination therapies.[61-64] Carmeli and colleagues[62] examined the emergence of resistance among the antipseudomonal agents ciprofloxacin, ceftazidime, imipenem and piperacillin. Emergence of resistance appeared during treatment with each class of antibacterial, as confirmed by genotypic analysis; combination therapy with an aminoglycoside did not appear to significantly prevent the emergence of resistance. Similarly, in a case-control study of *P. aeruginosa* bacteraemia, El Amari et al.,[63] through multivariate analysis, found that monotherapy was associated with a 2.5-fold greater risk of subsequent resistance to the single antipseudomonal agent utilised ($p = 0.006$). However, this risk was not significantly decreased when compared with previous combination therapy (adjusted OR = 1.8 [0.55–5.6]; $p = 0.34$). In a meta-analysis by Paul et al.,[64] when examining sepsis in immunocompetent patients, the relative risks (RRs) favoured monotherapy over combination therapy with respect to bacterial superinfection (RR = 0.79 [0.79–1.06]) and bacterial colonisation (RR = 0.86 [0.63–1.17]). Also, in a meta-analysis of eight randomised controlled trials, β-lactam monotherapy was not associated with greater emergence of resistance (OR 0.90; 95% CI 0.56, 1.47) than the combination of a β-lactam and aminoglycoside.[61]

Contrary to these findings, some studies have shown a benefit with combination therapy.[65-68] Gerber et al.[67] found that the addition of ticarcillin to gentamicin suppressed the emergence of gentamicin-resistance subpopulations of bacteria in a neutropenic mouse infection model. Drusano et al.[65] evaluated the emergence of *P. aeruginosa* resistance in neutropenic guinea pigs after administration of suboptimal dosages of meropenem either alone or in combination with once-daily tobramycin. Even though the number of animals was small ($n = 38$), no resistant pseudomonal isolates developed in the 9 animals that received combination therapy, while 22 of 29 (76%) animals receiving monotherapy developed resistance. Through the use of an *in vitro* time-kill study, Drago and colleagues[66] demonstrated that the combination of either levofloxacin or ciprofloxacin with any one of numerous β-lactams, including cefepime, ceftazidime, imipenem, piperacillin, piperacillin/tazobactam, or the aminoglycoside amikacin, selected for resistance less frequently than when the antibacterials were given as monotherapy. In addition, through the use of an *in vitro* hallow fibre model, Tam et al.[68] recently showed that the addition of tobramycin to meropenem suppressed the emergence of resistance against a wild-type and AmpC stable derepressed *P. aeruginosa* isolates.

Currently, no pharmacodynamic studies are available that describe pharmacodynamic breakpoints for agents given in combination. No previous study has attempted to link the pharmacodynamic target attained to a measure of clinical or microbiological efficacy. The limiting factor is the difficulty in determining which pharmacodynamic parameter best describes microbiological efficacy. For instance, with the combination of a β-lactam and an aminoglycoside, β-lactams are time-dependent and $T > MIC$ describes efficacy, while aminoglycoside efficacy is improved by increasing the C_{max}/MIC; when used in combination it is difficult to determine which parameter best predicts efficacy. Clearly, future studies are needed to determine the true impact of combination therapy and the potential benefit of preventing resistance development.

Caution has been expressed with the application of the concept of an MPC to β-lactams, macrolides and aminoglycosides because the primary mechanisms of resistance involve either acquisition of foreign DNA, inactivating enzymes, target binding site alterations or induction/depression of β-lactamases. Therefore, the MPC might not simply be indicative of the organism's ability to spontaneously mutate. Specific examples include drug inactivation of β-lactams as a result of β-lactamase production by Gram-negative pathogens such as *E. coli*,[69] and plasmid-mediated acquisition of *erm* or *mef*-mediated resistance in macrolides against *S. pneumoniae*. Additionally, the inoculum effect may limit the va-

lidity of this method with bacteria that produce β-lactamases. At lower inocula (e.g. 10^5 cfu/mL required in standard MIC methodology), β-lactamases may not be of a sufficient quantity to yield resistance. However, at the 10^{10} cfu/mL that is suggested to determine the MPC, the overproduction of β-lactamases may hamper observation of any underlying relevant mutations. Future work is needed to determine if this concept has validity with antibacterials other than fluoroquinolones.

3.3 Aminoglycosides

No studies have been performed to specifically evaluate the ability of aminoglycoside monotherapy to prevent the development of resistance. However, it is unlikely that studies will be performed in the future because of the risk of treatment failure when treating infections, such as bacteraemia, with aminoglycoside monotherapy.[70]

4. Conclusion

As a result of the dramatic increase in antimicrobial resistance and the limited number of new antibacterials on the horizon, finding the most effective way to administer the currently available antimicrobials is of paramount importance. Pharmacodynamic investigations have determined these regimens for many antibacterials, including the β-lactams, aminoglycosides and fluoroquinolones. Pharmacodynamic breakpoints must be targeted with optimal dosing regimens to maximise the potential of achieving clinical and/or microbiological cure. Studies specifically identifying pharmacodynamic targets that prevent the development of resistant mutants are beginning to be presented. It appears, at least for fluoroquinolones, that AUC/MIC exposures much higher than originally determined for eradication are necessary to prevent the emergence of a resistant subpopulation in both Gram-positive and -negative bacteria. Studies are needed to fully characterise whether specific targets can be achieved with currently used members of the various classes of antibacterials.

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