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Conserving antibiotics for the future: New ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective

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ABSTRACT

There is a growing need to optimize the use of old and new antibiotics to treat serious as well as less serious infections. The topic of how to use pharmacokinetic and pharmacodynamic (PK/PD) knowledge to conserve antibiotics for the future was elaborated on in a workshop of the conference (The conference "The Global Need for Effective Antibiotics – moving towards concerted action", ReAct, Uppsala, Sweden, 2010). The optimization of dosing regimens is accomplished by choosing the dose and schedule that results in the antimicrobial exposure that will achieve the microbiological and clinical outcome desired while simultaneously suppressing emergence of resistance. PK/PD of antimicrobial agents describe how the therapeutic drug effect is dependent on the potency of a drug against a microorganism and the exposure (the concentration of antimicrobial available for effect over time). The description and modeling of these relationships quantitatively then allow for a rational approach to dose optimization and several strategies to that purpose are described. These strategies include not only the dosing regimen itself but also the duration of therapy, preventing collateral damage through inappropriate use and the application of PK/PD in drug development. Furthermore, PK/PD relationships of older antibiotics need to be urgently established. The need for global harmonization of breakpoints is also suggested and would add efficacy to antibiotic therapy. For each of the strategies, a number of priority actions are provided.

1. Introduction

In an era of increasing emergence of drug resistance and lack of new antibiotics there is a growing need to optimize the use of old and new antibiotics to treat infections. Although the efficacy of new antimicrobials and dose–response relationships is reasonably described, this is often not the case for older agents. Much progress has been made over the past two decades in elucidating exposure–response relationships of antimicrobials, particularly regarding pharmacokinetic (PK) and pharmacodynamic (PD) principles. Perhaps even more important is the increasing awareness that optimizing therapy not only involves maximizing therapeutic outcome but also includes minimizing the risk of resistance emerging during therapy, both in the infecting pathogen and in the normal flora. However, the exposure–response relationships for efficacy and resistance selection are often distinctly different. Optimizing outcome is directed at the individual patient level whereas emergence of resistance is an ecologic issue and a trade-off between these two objectives is not always easy to achieve. In any event, it is essential that clinical decisions be based on exposure–response relationships. In some instances, this information is readily available but is not implemented; in many more cases, specific research is warranted. The knowledge obtained from further research should provide the tools for pol-

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icy changes but may also facilitate implementation of existing guidelines.

We will first discuss exposure–response relationships in general and provide the background of the PK/PD principles that can be used to optimize antimicrobial therapy (Section 2). These principles will serve as the backbone for a number of topics that are subsequently highlighted and involve the use of PK/PD in optimizing the use of antimicrobial agents: development of antimicrobials (Section 3), emergence of resistance (Sections 4 and 5) and the use of PK/PD in establishing and revising breakpoints for old and new antimicrobials (Section 6). Each of these topics concludes with statements that should improve the use of antimicrobials and indicate where research is needed in that specific area.

2. Background of PK/PD and exposure–response relationships

PK/PD of antimicrobial agents describes the triangular relationship between the potency of a drug against a micro-organism, subject exposure to a drug (the concentration of antimicrobial available for effect over time) and drug effects (Fig. 1). This relationship is somewhat different from that for non-antimicrobial drugs because the receptor of an antimicrobial drug is located within the microorganism instead of on a cell in the human body. Thus, the intended beneficial effects on the host will be secondary to the killing or growth inhibition of the pathogen. In this view, antimicrobial therapy is only one of the factors contributing to curing patients, albeit a significant one for most infections. Dosing regimen optimization is accomplished by choosing the dose and schedule that results in an exposure that will achieve the primary aim of the therapy (i.e. clinical outcome, resistance suppression or a specific degree of microbiological effect).

2.1. Effects of exposure

To determine the optimal exposure it is necessary to have a quantitative measure. The measure most often used is the area under the time-concentration curve (AUC) over 24 h (AUC_{0-24h}) (Fig. 2). The AUC can be regarded as the integration of the concentration over time and thereby represents the 'total' exposure of the antimicrobial during the period indicated and is expressed in (concentration × time) units (Mouton et al., 2005). One of the characteristics of the AUC is that it is, for many drugs, correlated to dose in a linear fashion. Thus, for example, an increase of the dose with a factor of 2 will yield an AUC that is twice as large. Similarly, applying the same dose twice will also result in an AUC that is twice as large, although for drugs with a relatively long half-life, accumulation affects this correlation during the first days of therapy.

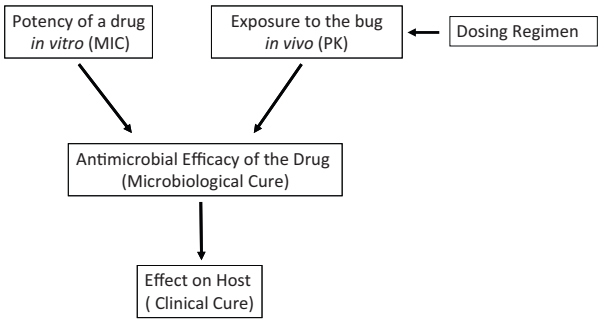

Fig. 1. Triangular relationship between the potency of a drug against a microorganism (usually expressed as a MIC), exposure of an antimicrobial drug (concentration of antimicrobial available for effect over time) and antimicrobial drug effects. The exposure of the drug is dependent on the pharmacokinetic properties of the drug and the dosing regimen.

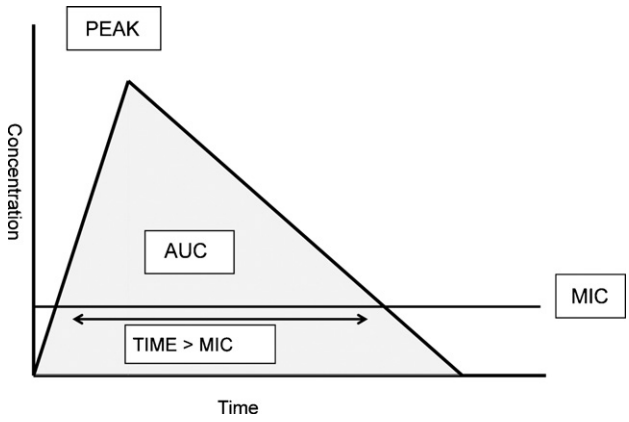

Fig. 2. Concentration–time curve showing the pharmacokinetic parameters peak (or C_{max}) and area under the time–concentration curve (AUC) (shaded area) and the pharmacodynamic index $T_{>MIC}$.

Exposure–response relationships have been studied in various *in vitro* experimental systems as well as in other hosts than humans. The primary purpose of these studies is to determine the exposures resulting in certain effects and subsequently deduce the optimal exposures needed for cure. Typically, this relationship is studied in animal systems where the neutropenic thigh model and pneumonia model in mice are the ones most commonly used. In these models, mice are rendered neutropenic and commonly infected with a specified inoculum of 10^6 bacteria in the thigh or lung. Treatment is then initiated and after 24 or 48 h the total bacterial count is determined for each organ. Using different doses and dosing intervals, ranges of exposure are obtained and subsequently plotted to the number of colony forming units (CFU) yielding exposure–response relationships. An example is depicted in Fig. 3, which shows the effect of different doses of levofloxacin in neutropenic mice with a pneumococcal lung infection (Scaglione et al., 2003). It is apparent that for relatively low AUCs virtually no effect is observed (outgrowth of bacteria to 10^8 CFU), whereas for high values there is a significant effect (decline to 10^2 CFU). The relationship can be described by a sigmoid curve. Notably, since the drug pharmacokinetics in mice differ from those in humans, the dose-response relationships will be markedly different, whereas the exposure–response relationships will be similar. The latter has been demonstrated in a number of studies and summarized recently (Ambrose et al., 2007). Ambrose and colleagues showed that exposures required for certain responses in preclinical models correlated well with exposures required for cure in humans.

However, except for a few early investigators, it was not fully appreciated until two decades ago that it is not only the total daily

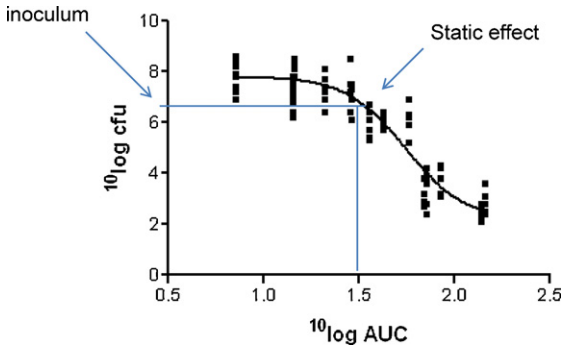

Fig. 3. Exposure (area under the time–concentration curve, AUC)–response (colony-forming units, CFU) relationship of levofloxacin and *S. pneumoniae*. Vertical line indicates the AUC required for a static effect, i.e. no net change in CFU after 24 h of treatment. After data in Scaglione et al. (2003).

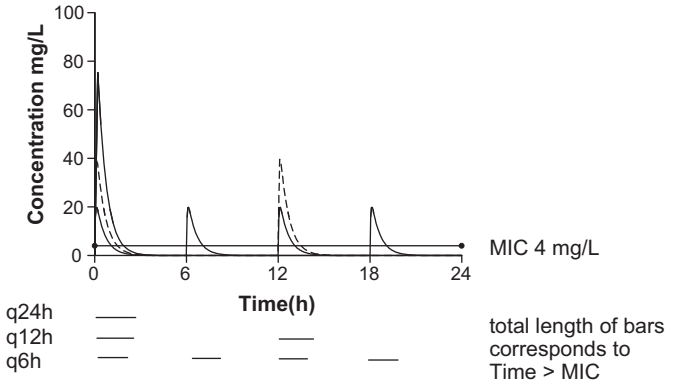

Fig. 4. Diagram showing that the $T_{>MIC}$ increases while the AUC remains the same if daily doses are divided. The length of the bars beneath the figure corresponds to the $T_{>MIC}$. For some antimicrobials (e.g., beta-lactam antibiotics), it is the $T_{>MIC}$ that is primarily correlated to effect.

dose, and thus 24-h exposure that determines outcome, but also the frequency of dosing (Fig. 4) (Eagle et al., 1950; Leggett et al., 1989). Whereas for most classes of drugs the AUC is correlated to effect, it has been shown that the efficacy of beta-lactam antibiotics is more dependent on the time the concentration of the antimicrobial remains above the minimal inhibitory concentration ($T_{>MIC}$) of the microorganism than on the AUC (Craig, 1998). Consequently, it is both exposure itself and the shape of the concentration–time curve, and thereby the frequency of dosing, that determine outcome. A more extended description of these relationships can be found elsewhere (Craig, 1998; Drusano, 2004).

2.2. Effects of the minimal inhibitory concentration (MIC)

As stated above and indicated in Fig. 1, the efficacy of an antimicrobial is dependent on exposure as well as its potency against the microorganism. The potency is usually expressed as a MIC. For antimicrobials in which the effect is AUC-dependent, there is a relationship between exposure, MIC and response in the sense that the response is dependent on the ratio between exposure and potency, or AUC/MIC. Fig. 5 shows the survival of four groups of rats infected with isogenic *Pseudomonas* strains with different MIC values and treated with varying doses of the quinolone lomefloxacin (Drusano, 2004; Drusano et al., 1993). The two groups with different MIC and AUC values (expressed as dose) but the same AUC/MIC ratio display the same response.

This principle is further demonstrated by a patient study illustrated in Fig. 6 (Rodriguez-Tudela et al., 2007). The figure shows the probability of cure in 132 patients with oropharyngeal *candidiasis*. For each patient, an estimate was made of the AUC based on the dose received (doses varied between 50 and 400 mg). The MIC of the *Candida* strain was also determined. The AUC/MIC ratio was determined for each patient, resulting in seven groups of similar AUC/MIC values. The proportion of patients cured was then determined for each AUC/MIC group and plotted against the AUC/MIC ratio. It is apparent that for relatively low AUC/MIC ratios virtually no effect is observed, whereas the probability of cure for high ratios approaches 100%. The shape of the curve follows the typical sigmoid response pattern. Again, it has to be emphasized that there is a range of AUC and MIC values, but it is the ratio between them that determines the outcome. Thus, if a certain exposure required for a certain effect was known or established, the MICs that could be covered by that exposure can be derived from the relationship between AUC/MIC ratio and effect, and vice versa. Optimal dosing then follows from the pharmacokinetics of the drug (which vary from individual to individual)

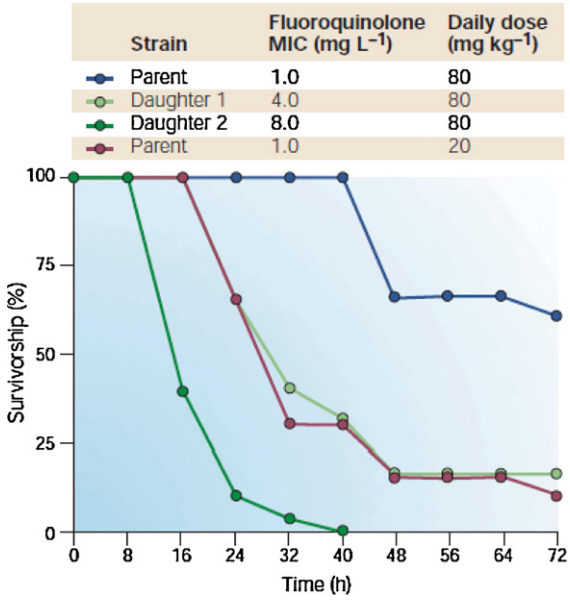

Fig. 5. Survival of four groups ($n = 20$) of neutropenic rats infected with 10^9 colony-forming units (CFU) per ml of three isogenic strains of *P. aeruginosa* treated with a fluoroquinolone (lomefloxacin) showing that survival of the groups (parent and daughter 1) with same AUC/MIC (AUC expressed as dose) ratio are similar. From Drusano (2004). Reproduced with permission from the publisher.

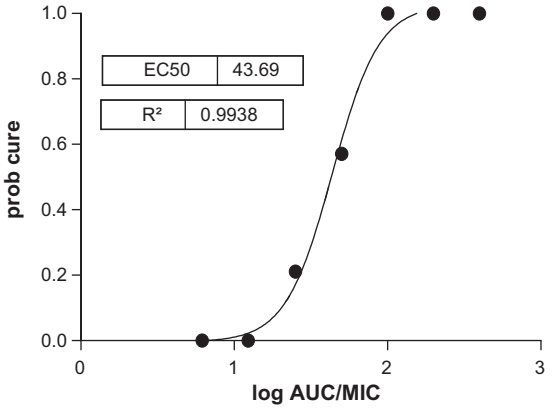

Fig. 6. Exposure–response relationship of fluconazole in patients with *oropharyngeal candidiasis* (Rodriguez-Tudela et al., 2007). Each data point represents the proportion of patients cured within a group representing a certain area under the time-concentration curve (AUC)/minimal inhibitory concentration (MIC) value. Reproduced with permission from the publisher.

and the MICs of the microorganisms (which vary from strain to strain).

3. Dose finding for new and old antibiotics

The classical phases of development of drugs involve phase 1, 2 and 3 studies before registration and phase 4 after registration or marketing authorization. Briefly, phase 1 studies involve escalating doses to establish the pharmacokinetic properties of the drug and detect possible toxic properties and other side effects. Phase 2 mainly focuses on confirming appropriate dosing regimens while in phase 3 the efficacy of the drug is established in comparative clinical trials. Phase 4 includes post-marketing surveillance, mainly to uncover side effects that are relatively uncommon and could therefore not have been detected in the earlier phases involving only a limited number of patients. Antimicrobials are different from other drugs because the final receptor is situated in the microbe and the

effect of the drug, and thereby the exposure–response relationships, involve the microorganism rather than the effect on human physiology. This allows the exposure–response relationships to be studied to optimize the dose for new and old drugs in experimental systems. Translating these relationships to treatment of infections in humans may then provide a tool to change the way antimicrobials are evaluated and approved.

3.1. Dosing regimen determination for new antibiotics

Because PK/PD describe exposure–response relationships, it follows that the response could be predicted and the optimal exposure for cure designed. The pharmacokinetics of the drug allow the derivation of a dosing regimen that should result in the desired exposure and is increasingly being used in antimicrobial drug development. It allows a rational choice to be made between drug candidates and supports determination of doses and exposures in phase 3 studies. This process involves several steps, starting with a description of the exposure–response relationship. As has been argued, this can be done in animals and in *in vitro* studies. From the results of these studies the target exposures needed for the microorganisms in question can be readily derived. The pharmacokinetic characteristics of the drug follow from the phase 1 studies and can be used to determine the required doses to achieve the desired exposure. An important issue here is the variation of pharmacokinetic profiles in the patient population. When a certain PK/PD target index (e.g., AUC/MIC ratio) is desired for every individual within the population, this should be true not only for the population mean but also for the part of the population with a higher elimination rate and thus lower than average exposure. To that end, Drusano et al. suggested an integrated approach of population pharmacokinetics and microbiological susceptibility information by applying Monte Carlo simulations (Drusano et al., 2000, 2001). This method takes the variability of the input variables into account and generates slightly different pharmacokinetic parameter values concordant with the variation in the population (Bonate, 2001). Thus, PK/PD index values are generated for both the population mean and every possible individual in the population. The population distribution of these index values is then used to estimate the doses needed to determine optimal exposures in the population, including those individuals with a high elimination rate. This approach has been used by several authors (Ambrose and Grasela, 2000; Bhavnani et al., 2005; Mouton et al., 2004), including for setting and evaluating clinical breakpoints, as well as establishing doses in phase 2 and 3 trials (Ambrose, 2006; Mouton, 2003). In conclusion, dosing regimens in phase 2 and 3 trials should ideally be based on preclinical PK/PD studies indicating potential pharmacodynamic targets that ascertain a high probability of microbiological cure. The data from phase 1 pharmacokinetic studies indicate the exposure of the antimicrobial after administration of the drug to humans. Thus, the extent of studies in humans to determine dosing regimens (phase 2) and large comparative trials (phase 3) could be reconsidered. Presently, many clinical trials are labeled phase 2/3 and carried out as comparative trials. We should use these studies to confirm the predicted efficacy based on PK/PD while simultaneously getting a reasonable indication of major safety concerns. Side effects that occur at a relatively low frequency need to be uncovered by exposure to (far) more patients than would be possible before market authorization and postmarketing surveillance would be more suitable to that purpose. These comparative trials also need to demonstrate that the antimicrobial effects of treatment by the new agent are not inferior to agents already available and using PK/PD tools may be more suitable to that end. Finally, PK/PD should predict the effect for less susceptible microorganisms. This approach will ultimately pose less risk to patients, increase the probability of effectiveness, determine a dosing regimen optimal for patient care and be less likely to result in resistance development (see below).

3.1.1. Suggested priorities

- During drug development and approval processes
  - Use PK/PD principles and tools when developing dosing regimens for clinical trials and setting breakpoints.
  - Develop methods using PK/PD to increase the power of comparative trials and (thereby) reduce the number of subjects in the studies.
  - Expand post-marketing surveillance (phase 4) to increase detection of adverse effects.

3.2. Dosing regimen determination for old antibiotics

In the past, antimicrobial agents were developed more on a trial and error basis and many were licensed before controlled clinical trials became mandatory (Podolsky, 2010). Accordingly, for these drugs, the information to optimize dosing regimens using exposure–response relationships is not readily available, if at all, and it is unclear whether the current dosing regimens used are optimal or even efficacious. Even if comparative trials were performed in the past to determine whether one antibiotic was non-inferior or superior to another, the dosing regimens are often changed over time. These changes in dosing regimens pose a significant problem because old off-patent antibiotics are increasingly being prescribed to patients now that emerging resistance creates an increasing challenge in antimicrobial treatment of Gram-negative bacteria in particular. In many cases microorganisms are now fully resistant to commonly used drugs and some of these old agents are used as a last resort. Examples include extended-spectrum-beta-lactamase (ESBL) producers, and recently, KPC (*Klebsiella pneumoniae* carbapenemases) or NDM-1 (New Delhi Metallo-beta-lactamase) producers (Hammerum et al., 2010). Old drugs, such as colistin and fosfomycin, must then be used without any certainty that the correct dosing regimens are being applied (Lim et al., 2010). A re-evaluation of these drugs is urgently needed, including establishing PK/PD relationships and the optimal dose.

3.2.1. Suggested priorities

- Obtain exposure–response relationships for old antimicrobials.
- Develop criteria to re-evaluate approval and indication of all antimicrobials presently available, prioritizing those older agents required for the management of multiresistant organisms.
- Establish a mandatory process to re-evaluate indications and dosing regimens of antimicrobials. Market authorization should be awarded for a limited time period (e.g. 5 years) instead of granting unlimited duration.

4. Exposure–response relationships and emergence of resistance

In the previous section a quantitative description was given regarding the relationship between exposure intensity (e.g., the AUC/MIC ratio) and efficacy. An important characteristic of this relationship is that it is sigmoid and monotonic (Figs. 3 and 6). That is, at very low values of exposure intensity, there is no measurable effect, whereas at larger values, the greater the exposure intensity, the greater the bactericidal effect up to a maximal value. For suppression of resistance selection during treatment, this is *absolutely* not the case. Here, the relationship between exposure and resistance selection is distinctly non-monotonic and has the shape of an inverted "U". Tam and colleagues demonstrated this relationship

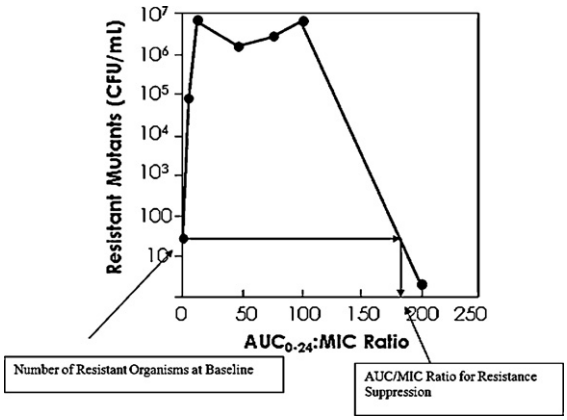

Fig. 7. Changes in a quinolone-less susceptible subpopulation as a function of the area under the time–concentration curve (AUC)/minimal inhibitory concentration (MIC) ratio (Tam et al., 2007b). Reproduced with permission from the publisher.

for several strains of bacteria in a mouse thigh model and also in a hollow fiber infection model (HFIM), where the effect of a quinolone against *P. aeruginosa* was investigated (Tam et al., 2005, 2007b). At the end of the experiment, the size of the resistant subpopulation was plotted against the AUC/MIC ratio. The first data point is the number of resistant colonies at baseline before therapy initiation (Fig. 7). As can be seen in the figure, even small exposures cause considerable amplification of the resistant subpopulation. Ultimately, a maximal value is attained after which increased exposure causes a decline in the number of resistant colonies towards baseline. The horizontal line in Fig. 7 demonstrates the regimen intensity required to return the number of resistant colonies to baseline (AUC/MIC ratio circa 190). Other investigators have found similar relationships (Firsov et al., 2003; Stearne et al., 2007). The markedly increased intensity required for resistance suppression compared with the exposure required for efficacy is important. Until now, most dosing regimens have been optimized for efficacy, but the shape of the curve in Fig. 7 indicates that the values required for efficacy may amplify resistant subpopulations. Thus, it is important to identify an exposure (and thus dose) that suppresses resistance as well as provides a good bactericidal effect.

Whereas the general relationship between exposure and emergence of resistance can be described by an inverted U-shaped pattern, there are three factors that generally have an impact on the value of the maximum and exact shape of the curve: The first is the number of bacteria present or the inoculum size in experimental settings. The second is the duration of therapy and the third is the presence and activity of an immune system.

4.1. Inoculum size

Jumbe et al. (2003) examined the effect of levofloxacin against *P. aeruginosa* in a granulocyte-replete mouse thigh infection model. They first demonstrated (Fig. 8) that there was a relationship between regimen intensity (indexed to AUC/MIC ratio) and the ability to kill microorganisms at the primary infection site. Subsequently, they showed that this relationship was markedly affected by the initial inoculum size (Fig. 8: panel a vs. panel b). In panel a, the challenge was 10^6 bacteria and in panel b 10^7 bacteria. The established mutation frequency was lower than 1 in 10^6 and higher than 1 in 10^7 for the strain used. After a 2-h lag, therapy was initiated. The difference in the size of the inoculum resulted in a 2–5-fold difference in the exposure intensity required to attain the same antibacterial effect. This difference occurs because in panel a there is a single susceptible population, whereas in panel b there are two populations, a susceptible one and a less susceptible one

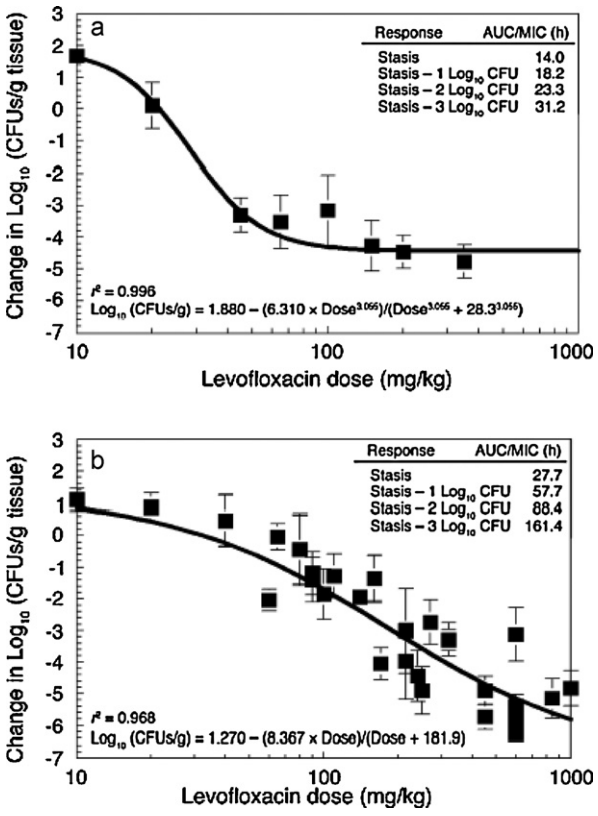

Fig. 8. (a) Exposure–response of *Pseudomonas aeruginosa* bacterial cell kill as a function of levofloxacin exposure. A challenge of 10^6 colony-forming units (CFU) was employed. (b) Exposure–response of *P. aeruginosa* bacterial cell kill as a function of levofloxacin exposure. A challenge of 10^7 CFU was employed.
From Jumbe et al. (2003). Reproduced with permission from the publisher.

(the resistant mutants). The latter population (i.e. the less susceptible one) responds less well to antimicrobial therapy. Jumbe et al. (2003) also employed a complex mathematical model to analyze all the data simultaneously, calculating the exposure necessary to suppress resistance emergence from the model parameters. In a prospective evaluation two regimens were studied: one predicted to amplify resistant subpopulations and one predicted to suppress resistant subpopulations. The total population and resistant subpopulation are displayed in Fig. 9 together with their response to the two regimens (panels a and b). The lines are prospective prediction lines rather than best-fit lines. Clearly, the regimens behaved exactly as predicted and indicate that the degree of exposure – here expressed as an AUC/MIC ratio – is a tool that we may employ to help suppress resistance emergence. It is critical to apply this insight to our currently available drugs to prolong their useful lifespan. It is, perhaps, even more imperative to apply this principle to new drugs currently under development in order to slow down the cycle of drug development/resistance emergence. However, it is important to note that this is just one example; relationships may be different for different classes of drugs and the mutation frequency is variable.

4.2. Duration of therapy

Another simple principle is that the longer therapy continues, the more difficult it is to suppress amplification of a resistant subpopulation. A regimen that only lasts for 4–5 days may provide good bactericidal effect and be adequate to minimize amplification of a resistant mutant subpopulation. However, extending that regimen to 10 days may cause therapy failure by resistance emer-

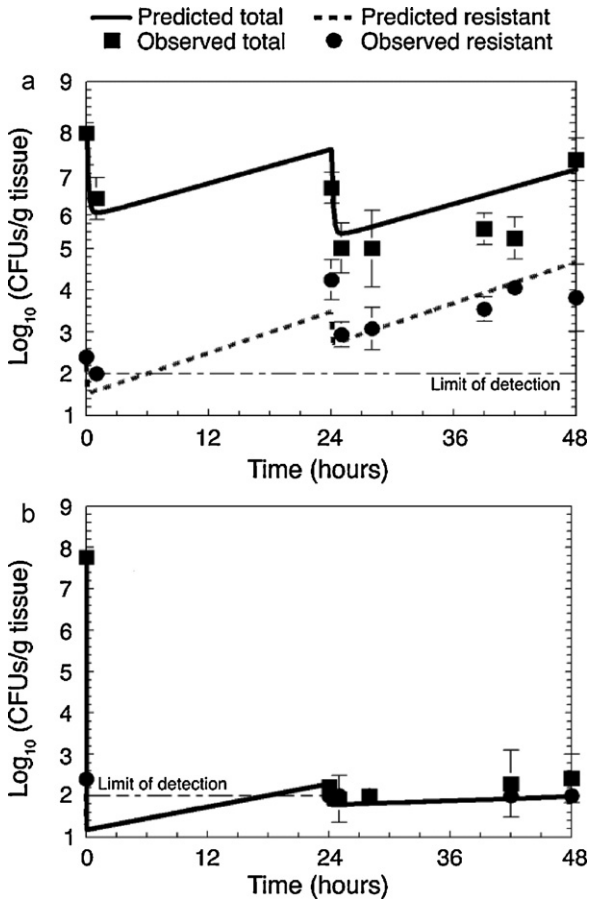

Fig. 9. Prospectively predicted (lines) and observed responses of *Pseudomonas aeruginosa* total population (solid line and squares) and levofloxacin-less susceptible population (dashed line and circles). Panel a: a regimen designed to amplify the less susceptible population (area under the time–concentration curve (AUC)/minimal inhibitory concentration (MIC) ratio = 53). Panel b: a regimen designed to suppress the less susceptible population amplification (AUC/MIC ratio = 157).
From Jumbe et al. (2003). Reproduced with permission from the publisher.

gence if not all bacteria were killed. Tam et al. (2007a) examined the effect of garenoxacin against *S. aureus*. The authors evaluated two regimens: based on extensive mathematical modeling; one regimen with an AUC/MIC ratio of 100 and one with a ratio of 280. The lower intensity regimen was chosen to suppress resistance amplification for 4–5 days while the more intense regimen was chosen to suppress resistance amplification for a full 10 days. Of interest, the regimens were predicted to provide the same maximal kill rate for 4–5 days. The result is shown in Fig. 10, panels a and b. In panel a, the total population is displayed. As prospectively predicted from the mathematical model, both regimens have exactly the same 5-day kill rate. After this period, however, the less intense regimen ceases to be effective. In panel b, we can see that this failure is due to amplification of the resistant subpopulation. If therapy had been ended at day 4 or 5, little or no resistant mutant amplification would have occurred. This point was proven in a subsequent publication by Drusano et al. (2009a) where the behavior of the susceptible and resistant populations was studied after the drug pressure had been withdrawn. Briefly, the susceptible population took over and demonstrated that regimens should be very intense to obtain maximal bactericidal effect and to suppress resistance. In addition, regimens should be as short as possible in order to maximally suppress resistance

4.3. Effect of the immune system

The effect of exposure on emergence of resistance has also been studied in the HFIM. In this model, no immune system exists and microorganisms will re-grow in the absence of antibiotic pressure unless the whole population is eradicated. In contrast, in real clinical life most patients have a functional immune system; in particular, patients have granulocytes that contribute to bacterial kill. Drusano et al. (2010) recently demonstrated that, depending on the species, granulocytes can eradicate microorganisms up to about 10^5–10^6 CFU/g. For *S. aureus* and *P. aeruginosa*, granulocytes can kill up to 50 (CFU/g) per day. Consequently, if the antimicrobial treatment drives the total population of the organism down to around 10^2–10^3 CFU, as was done in Fig. 10, it is highly likely that terminating therapy after 5 days will allow the residual population to be eradicated by the immune system with minimal amplification of resistant mutants. Retaining a functional immune system is consequently instrumental in reducing emergence of resistance.

4.3.1. Suggested priorities

- Promote strategies for early treatment to reduce the increment of the infectious microorganism and maximize the antibacterial effect.
- Prevent underdosing to suppress or decrease the potential amplification of resistant mutant subpopulations.

5. Modifying exposure–response relationships to prevent emergence of resistance

Resistant bacteria may emerge during therapy and from a clinical perspective, despite the doubtless benefits of antimicrobial agents, their intense use over the years has resulted in selection for resistance against these compounds in bacterial populations (Sykes, 2010).

It is widely accepted that once a bacterial population becomes resistant, either by mutation or by acquisition of resistance genes, it tends to persist. Resistance may be spread to or amplified in different bacterial populations, including those in nosocomial and community settings. In addition, resistance genes may be transferred to other susceptible populations (Livermore, 2005). Resistant organisms may accumulate several mechanisms of resistance, creating multi-resistant, extensive resistant or pan-drug resistant organisms for which few or no antimicrobials are currently available (Souli et al., 2008). Some of these organisms have become epidemic even in the community, where selective pressure may theoretically be lower.

From the sections above, it is obvious that a relationship exists between the pattern of exposure and emergence of resistance. Even more important, this relationship has also been described quantitatively and therefore provides the possibility to design dosing regimens that prevent or at least decrease the probability of resistance emergence or spread. These designs are based on hypothetical dosing regimens leading to a decrease in resistance emergence while retaining activity and have also been verified to actually work. Since the relationship between emergence and resistance follows an inverted U-shape as discussed in Section 4, it follows that there are two basic strategies; the first being a decrease and the second an increase in exposure. Unfortunately, neither of these strategies is applied as much as one would wish for, particularly in reducing exposure. Indeed, problems with the irrational use and of antibiotics and thereby unnecessary overexposure have been widely described (Gyssens, 2001; Harbarth and Samore, 2005).

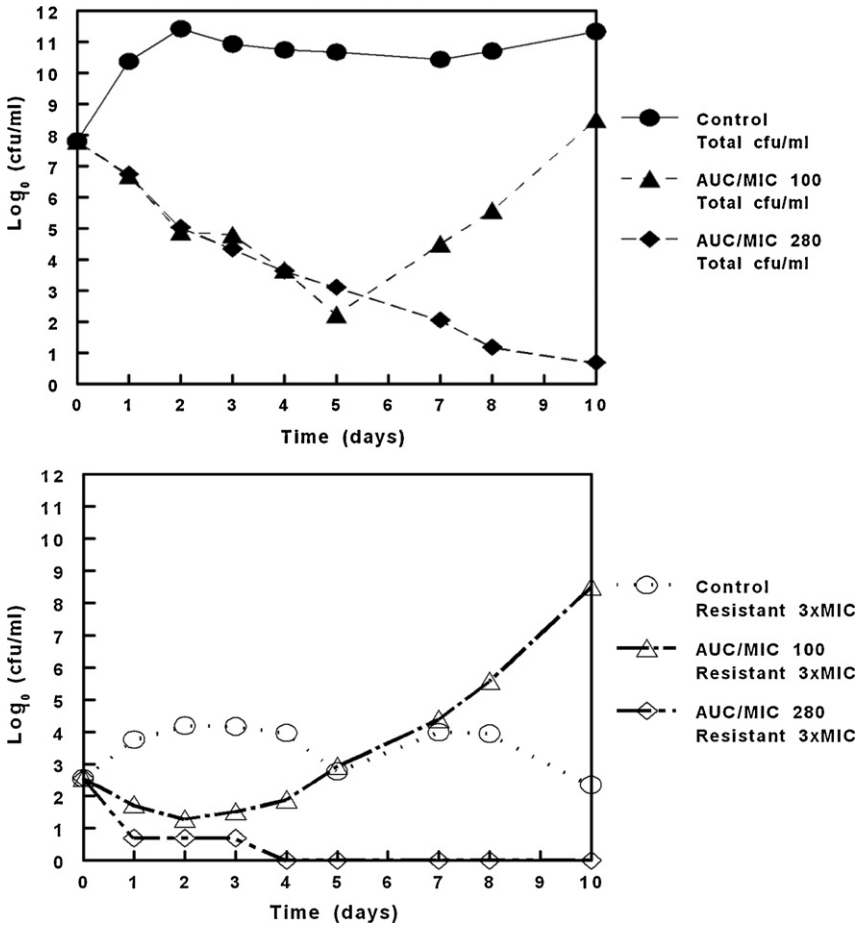

Fig. 10. (a) Impact on the total population of *Staphylococcus aureus* over time by two regimens of garenoxacin. (b) Impact on the less-susceptible population of *S. aureus* over time by two regimens of garenoxacin.
From Tam et al. (2007a). Reproduced with permission from the publisher.

5.1. Reducing exposure by reducing the duration of therapy and prophylaxis

In addition to reducing the unnecessary use of antibiotics, one of the simplest and most effective ways to reduce exposure is to shorten courses of antibiotic treatment and prophylaxis. With rare exceptions (e.g. bacteremia due to *S. aureus,* endocarditis, osteomyelitis), there is no evidence to support most of the traditional 10–14-day courses of antibiotics, which are based more on conventional wisdom than strong evidence. Short-course therapy for urinary tract infection, acute otitis media, tonsillopharyngitis, sinusitis and pneumonia is slowly gaining support (MASTIN study group, 2002; Lutters and Vogt, 2002). The short course paradigm is, in principle, widely extensible to the broad range of antibiotic prescriptions (including perioperative antibiotic prophylaxis) used by surgeons in both hospital and ambulatory settings. However, there is an ongoing problem of unnecessary prolongation of perioperative antibiotic prophylaxis (Bratzler et al., 2005; Dettenkofer et al., 2002; Huskins et al., 2001) despite extensive evidence arguing in favor of short course or even single dose administration (Saxer et al., 2009). A large cohort study demonstrated that extended antibiotic prophylaxis after cardiovascular surgery did not decrease the risk of surgical site infection but instead increased the risk of carriage of antibiotic-resistant pathogens (Harbarth et al., 2000).

An important barrier to rational antibiotic use and decreased treatment duration is the lack of efficient and affordable diagnostic tools with high sensitivity and specificity to distinguish bacterial from viral diseases. Few biomarkers are available to guide antibiotic treatment and duration decisions. Procalcitonin is the best studied of these markers and in several controlled clinical studies, it has shown to be useful in reducing inappropriate use of antibiotics and guiding duration of treatment (Christ-Crain et al., 2006; Harbarth et al., 2009). The need for development of new diagnostic tools is discussed in greater detail in another article in this issue (Okeke et al., 2011).

Among available strategies to decrease antibiotic usage, reductions in duration of antimicrobial treatment are the safest and likely to be the most palatable to practicing clinicians (Rice, 2008). More studies are needed, however, to define minimal lengths and maximal doses of therapy to ensure that efforts at reduced use are safe and effective.

5.1.1. Suggested priorities

- Implementation of short-course therapies based on both preclinical data (Section 4) and available evidence from prospective studies.
- Implementation of perioperative antibiotic prophylaxis guidelines.
- Development and use of diagnostic tools to reduce inappropriate use of antibiotics and length of therapy.
- Randomized controlled studies to define the optimal duration of therapy.

5.2. Reducing exposure by cycling and sequential therapy

Interventions targeted at reducing selection pressure via scheduled repetitive cycling of different classes of antibiotics on wards or in institutions have been pursued in attempts to control the emer-

gence of antibacterial resistance locally. Typical cycling protocols use periods of one to several months. The theoretical benefit of cycling primarily rests on the assumption that resistance affects only single antibiotics or antibiotic classes and that resistant bacteria are less fit and will have a growth disadvantage upon withdrawal of the selective antibiotic pressure. Resistance should then decrease during periods of non-exposure, which would justify cycling protocols. However, reported clinical effects of cycling remain inconclusive for two primary reasons: methodological flaws undermine published intervention trials (Brown and Nathwani, 2005; Nijssen et al., 2006) and, far more troubling, the evolution of bacterial multidrug resistance in health care settings has outpaced our assumptions.

Many resistant bacteria are commonly not less fit with compensation of fitness through additional mutations (Schulz zur Wiesch et al., 2010). Consequently, it should be no surprise that a decline in resistance has not been observed in response to reduced usage through the cycling periods. Furthermore, in locations with a high prevalence of multidrug resistance, unspecific resistance mechanisms (e.g. up-regulation of efflux systems) cause co-selection pressure for different classes of antibiotics affected by the same efflux system (O'Fallon et al., 2009). Any antibiotic classes or other compounds that are substrates of the efflux system (such as triclosan) maintain selection pressure during cycling periods (Chuanchuen et al., 2001). Because *P. aeruginosa* has several efflux pumps, it is a typical example of a pathogen that may not be affected by cycling (Tsukayama et al., 2004). Mobile genetic elements, which carry several unrelated resistance determinants and have been noted with increasing frequency, also contribute to co-resistance. The linkage of ESBL/carbapenemases, aminoglycoside modifying enzymes and quinolone resistance genes on transferable mobile genetic elements in enterobacteria and *Acinetobacter* is specifically relevant and frequent (Mak et al., 2009; Miro et al., 2010; Mooij et al., 2009; Vinue et al., 2010). It has been suggested and confirmed from clinical experience that such Gram-negative bacteria may not respond to cycling strategies (Raineri et al., 2010).

Cycling exposes patients to high homogenous selection pressure against employed antibiotics, potentially extending to other classes with associated co-resistances. Thus, cycling selection pressure possibly promotes the development of resistance within short periods facilitating outbreaks of multidrug resistant bacteria (Damas et al., 2006; Hedrick et al., 2008; Meyer et al., 2009; Nijssen et al., 2010; van Loon et al., 2005). Additionally, mathematical modeling corroborates the limited success reported thus far from clinical trials of antimicrobial cycling (Bergstrom et al., 2004). In summary, there is little evidence, empirical or theoretical, that cycling of homogeneous antibiotic exposure controls the emergence and spread of antibiotic resistance (Kollef, 2006; Sandiumenge et al., 2006; van Loon et al., 2005). Therefore, this intervention should not be implemented as a routine standard protocol (Brown and Nathwani, 2005; Levin and Bonten, 2004).

On a patient level, sequential use of antibiotics and its impact on the emergence of resistance remains poorly described. Because chronic and recurrent infections carry a high risk of emergence of resistance, cycling or sequential usage of different antibiotic classes may influence resistance. With the exception of *Helicobacter pylori* infections (Gisbert et al., 2010), no available clinical studies provide corroborating evidence. In addition, mathematical modeling also fails to support such regimens (D'Agata et al., 2008). IV-oral step-down therapy and de-escalation principles with a change to a reduced-spectrum antibiotic based on microbiological results are widely recommended aspects of antibiotic stewardship. Their effect on control of resistance emergence has yet to be quantified, however.

5.2.1. Suggested priorities

- Discourage the use of cycling schemes
- Perform further studies on the sequential use of antibiotics

5.3. Increasing exposure through combination therapy

The use of combinations of antimicrobial agents is common practice during clinical therapy, most notably for the treatment of severe infections and empirical therapy. The most accepted rationale for a combination antimicrobial therapy approach is an increase in the spectrum of coverage, even though current antimicrobials possess extremely broad activities. Assuming the pathogenic organism is susceptible to one antibiotic, the incremental benefit of combination therapy in the sense of synergistic activity is uncertain as evidenced by two recent meta-analyses (Paul et al., 2004; Safdar et al., 2004). In these studies, no significant difference in outcome was found between patients that received combination therapy vs. those that received monotherapy, except perhaps for infections caused by *P. aeruginosa*. One of the reasons that no significant difference in outcome was observed between the groups receiving monotherapy and combination therapy might have been diversity of patients and indications. Indeed, two other recent studies did demonstrate superiority of combination therapy for specific patient groups. In one meta-analysis, Kumar et al. (2010a) did not find an overall benefit, but when stratified for mortality, the group that showed the highest mortality did significantly better with combination therapy. In another study from the same authors, early combination antibiotic therapy yielded improved survival compared with monotherapy in septic shock (Kumar et al., 2010b). These studies show that for severely ill patients or patients with *P. aeruginosa* infections, combination therapy could be warranted. This observation is in line with studies that have looked at the effect of combinations in *in vitro* pharmacokinetic models and animal studies (den Hollander et al., 1997; Louie et al., 2010; Mouton et al., 1999b). These studies also show that the effect of combination therapy may be dependent on the resistance mechanism, i.e. with a similar phenotype in terms of MIC, the effect of the combination can be beneficial (Drusano et al., 2009b).

A specific topic is the use of combination therapy to minimize the risk of emergence of resistance. This has been demonstrated for the treatment of patients with AIDS and patients with tuberculosis, although the optimal exposures of the individual drugs and combinations have yet to be established (Lienhardt and Davies, 2010). For these disease entities, treatment with monotherapy is regarded as obsolete and even dangerous. If then, for the treatment of 'common' bacterial infections the risk of emergence of resistance is increasing for various reasons, it seems prudent to treat these infections with combinations of antibiotics, not only to increase the probability of cure but more so to retain activity of the antimicrobials. This is particularly true for those microorganisms that are known to become resistant during treatment, such as *P. aeruginosa* and other non-fermenting bacteria, which are ubiquitous in nature. Although it is difficult to show this benefit in clinical trials, there are several preclinical studies that clearly indicate that combination therapy in some instances may prevent the emergence of resistance (Louie et al., 2010; Mouton, 1999a). Resistant mutants usually occur at fixed frequencies (range 10^{-9} to 10^{-10}). However, under certain circumstances, especially during chronic infections such as bronchopulmonary infections in cystic fibrosis or patients with chronic obstructive pulmonary disease (COPD), resistant mutants can emerge at higher frequencies. This means, that even at low numbers, these populations contain bacteria with hypermutator phenotypes. These phenotypes are caused by mutations in DNA repair or error avoidance systems (mainly the mismatch repair system) (Blazquez, 2003; Chopra et al., 2003). Consequently, the probability to accumulate mutations in resistance

genes is higher, an event that leads to the emergence of resistant mutants at higher frequencies. Although hypermutators initially have lower fitness than wild-type strains, compensatory mutations can stabilize these populations and several mutations can accumulate leading to resistance to different antimicrobials (Harrison and Buckling, 2005; Oliver et al., 2000). Different approaches have been proposed to control hypermutators, but it is still an area of basic research. To some extent, their impact can be diminished with combination therapy (Oliver, 2010; Plasencia et al., 2007).

Specific attention must be paid to the use of old antibiotics in combinations. As stated above, old antibiotics (such as colistin) have been reintroduced as last resort therapies. However, they are also used (or are promoted to be used) in combination treatment. However, efficacy of these antibiotics in combinations has not been studied systematically and therefore it remains unclear whether combinations provide a clear benefit in these cases.

5.3.1. Suggested priorities

- Use combination therapy for severely ill patients.
- Use combination therapy for specific indications (e.g., *Pseudomonas* infections).
- Increase research to show benefits for specific indications.

5.4. Speaking the same language – defining clinical susceptibility

One of the most important issues over the past decade in the discussion on appropriate treatment and emergence of resistance is the 'language of resistance'. There are two important concerns related to the same issue: The first pertains to the methods used for susceptibility testing and the second to the interpretation of the test itself. At present, there is no international standard for routine susceptibility testing in either the human or non-human context. Serious progress was made in 2006 when the International Organization for Standardization (ISO) published a reference standard for the susceptibility testing of rapidly growing aerobic bacteria (ISO, 2006). This method sets the benchmark for two widely used methods, namely the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In an accompanying guideline it is described how susceptibility testing methods should be calibrated against the ISO standard. Unfortunately, there are still methods used worldwide that have not undergone a rigorous comparison with the ISO standard. The world will benefit greatly when there is complete harmonization of methods that show good correlation with the reference standard.

Perhaps an even more pressing problem is that the interpretive criteria differ worldwide, which is especially evident between CLSI and EUCAST. There are several reasons for these differences, mainly historical ones, but the fact remains that strains may be called susceptible using CLSI criteria and resistant using EUCAST criteria. A similar situation existed within Europe until a few years ago, where every country applied its own breakpoints; even worse, many laboratories used breakpoints as they saw fit. Whereas a harmonization process is under way in Europe and European harmonized breakpoints are available for use, not all European countries and laboratories have implemented this process. As a consequence, resistance rates still differ in Europe in part because of the interpretation used and not because of real differences. Importantly, many breakpoints still in use are considered too high by present day (EUCAST) standards and detection of resistance is therefore hampered while at the same time strains are categorized as susceptible, although infections caused by these microorganisms cannot be treated adequately. Two prominent examples show that if old breakpoints that are too high are used and strains are classified as susceptible, the probability of a fatal outcome increases (Tam et al., 2008) (Bhat et al., 2007). It has to be emphasized that PK/PD relationships readily predicted the outcome in both these studies and can therefore be taken as a validation for the application of pharmacodynamic principles in setting breakpoints. Whereas dosing and indications use to differ, which account for differences in breakpoints in the past, differences tend to disappear with the globalization and the ready dissemination of medical knowledge. It would therefore seem appropriate in the near future to harmonize breakpoints worldwide. Such an objective will require a long process and careful thought must be given on how best to accomplish this goal. To begin with, a world committee on antimicrobial susceptibility testing would be needed to set up and describe the process to accomplish this objective.

5.4.1. Suggested priorities

- Set up a committee to examine the pathway to harmonize breakpoints worldwide
- Provide expert guidance for clinicians to better understand breakpoints

5.5. Speaking the same language – defining resistance

Clinicians are primarily interested in susceptibility testing regarding treatment, and clinical breakpoints are set with that goal in mind. In contrast, epidemiologists and others involved in early detection of resistance are more interested in emergence of resistance as a mechanism. The presence of a resistance mechanism does not always mean that the microorganism (or rather patient) cannot be treated: if exposures following adequate dosing are high enough with respect to the MIC of the microorganism causing the infection in such a way that a near maximum effect can be reached (see Section 2), there is no reason not to use that agent. Clinical breakpoints are used in clinical laboratories and constitute the basis of their reports because they are primarily focused on guiding therapy. However, clinical breakpoints are clearly not designed for early detection of resistance or detection of resistance mechanisms. This point was recognized by the EUCAST when reassessing breakpoints in Europe (Kahlmeter et al., 2006). The EUCAST has therefore, apart from clinical breakpoints, defined wild-type (WT) distributions of bacteria (and fungi) that delineate the MICs of naturally occurring bacteria. The upper end of the WT distribution is demarcated by the epidemiological cut-off value (ECV). It is specific for each species and thus separates microorganisms without (wild type) and with (non-wild type) acquired resistance mechanisms to the agent in question. A microorganism with a value higher than the ECV is suspected of harboring a resistance determinant and these values can be used to monitor resistance development. However, until now ECVs have not been used on a wide scale for that purpose.

5.5.1. Suggested priority

- Implement the use of the epidemiological cut-off value (ECV) on a wider scale.

6. Concluding remarks

Exposure–response relationships have changed the way we look at the efficacy of antimicrobials and have provided us with a tool to design evidence-based dosing regimens. Although there is still a great deal of exploring to do and discoveries to be made, the present state of knowledge is now such that it can serve as a firm base for policy changes and their implementation. In this review, we have attempted to provide a number of priorities that need and can be acted upon relatively fast. Some of these priorities include the establishment of a committee or other working party to prepare the necessary policy changes. This and the other reports in this issue of Drug Resistance Updates are the result and compilation of presentations and discussions during the ReAct conference "The

Global Need for Effective Antibiotics–moving towards concerted action" in Uppsala 2010. We strongly believe that these reports can serve as an excellent starting point for the work of the proposed committee or working party.

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