What should be considered in the treatment of bacterial infections by multi-drug therapies: A mathematical perspective?

Francisco Pimenta^c, Ana Cristina Abreu^a, Lúcia Chaves Simões^{a,b}, Manuel Simões^{a,*}

^a LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

^b CEB, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

^c Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

ARTICLE INFO

Keywords:

Infections
Multi-drug therapy
Drug interactions
Resistance
PK/PD models

ABSTRACT

Bacterial infections are a global health concern with high levels of mortality and morbidity associated. The resistance of pathogens to drugs is one leading cause of this problem, being common the administration of multiple drugs to improve the therapeutic effects.

This review critically explores diverse aspects involved in the treatment of bacterial infections through multi-drug therapies, from a mathematical and within-host perspectives. Five recent models were selected and are reviewed. These models fall into the following question: which drugs to select, the respective dose, the administration period to effectively eradicate the infection in the shortest period of time and with reduced side effects? In this analysis, three groups of variables were considered: pharmacokinetics, pharmacodynamics and disturbance variables. To date, there is no model that fully answers to this issue for a living organism and it is questionable whether this would be possible for any case of infection.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The discovery of antibiotics in the twentieth century, along with the improvement of hygiene conditions, has significantly reduced the mortality associated to bacterial infections (Davies, 2006). However, infections are again a public health concern due to the growing number of microorganisms resistant to antibiotics. To contextualize the dimension of this problem, two numbers respective to the Europe Union are presented: the cost that antibiotic-resistant infections pose yearly is 1.5 billion €, with 25,000 associated deaths (Kirby, 2012).

In the development of new drugs, there may be a significant time lapse between the synthesis/discovery of a new compound and its approval and clinical use. Furthermore, antibiotics are usually derived from microbial metabolites (pure or modified) and half of the antibiotics currently in use were discovered along the antibiotics golden age, 1950–1960 (Davies, 2006), although novel approaches are underway (Coates and Hu, 2007). The mitigation of antibiotic resistance has been accomplished through the use of new antimicrobial compounds, by the combination of existing compounds (Boucher et al., 2013) and also through a more rational use

of antimicrobials (Bartlett et al., 2013). However, the combination of existing drugs seems to be a more rapid and accessible solution.

In the treatment of an infection by the combination of drugs – multi-drug therapy – it should be defined a set of parameters related to the strategy to be used: which drugs, in what concentration, and using which algorithm of administration (Fig. 1)? The selection of these parameters should ideally take into account several variables, which are classified in this work as: pharmacodynamics, pharmacokinetics, and disturbance variables (Fig. 1). This procedure is applied to achieve a set of established goals, such as to completely eradicate the population of pathogens (although resistance may emerge along the treatment, drug resistant bacteria should be eliminated to accomplish this goal; avoid drug resistance could also be a goal if epidemiologic considerations were applied, which is not the case) and to minimize the eradication time and side effects (renal effects, intestinal effects, cardiac effects, among others, according to McKinnon and Davis, 2004) (Fig. 1). Yet, a given therapy often requires a compromise between these three objectives, since fulfilling one may imply a deviation from the others. The challenge is to define a strategy that allows to the highest closeness of all three goals.

In Fig. 1, it is assumed that all the information needed on the infection to be treated is known. However, a detailed analysis of each individual case of infection is often impractical, whether due to the urgency raised by the situation (technological limitations) or by

* Corresponding author. Tel.: +351 225081654; fax: +351 225081449.
E-mail address: mvs@fe.up.pt (M. Simões).

Fig. 1. Infection treatment approach in a living organism, using a multi-drug therapy, from a mathematical perspective. A multi-drug therapy is defined by (A): the drugs to be used (including the route of administration), the respective concentration and the time at which each drug should be administered (as well as the therapy duration). The interaction established between both drugs (type and intensity) depends on the type and concentration of each drug. These three inputs of a therapy should be defined taking into account three groups of variables: (B1) pharmacodynamics (related with the effect of the drug on the pathogen), (B2) pharmacokinetics (related with the effect of the organism on the drug) and (B3) disturbance (these are variables that, although they can be modeled, there is a high uncertainty degree associated with them). Note that these variables are inter-associated (for instance, the non-compliance with the therapy may increase the level of resistance). The ultimate goal of the therapy is to attain (C) an effective (all pathogens are cleared) and efficient (side effects and clearance time are minimized) treatment of the infection. The scheme offers a holistic view of a therapy, although other topics could be added. Epidemiologic aspects were not considered in the present within-host analysis, although they are of utmost importance in a real clinical situation (the inclusion of epidemiology would have effects on the three groups of variable depicted in the figure).

the unavailability of material/economic resources. Thus, available information is generally limited and it is quite common to follow guidelines for drugs administration, which are based on established policies, apparent symptoms and in the patient past records (Deresinski, 2007; Houck et al., 2004). Some of these guidelines still follow the old and very popular: “hit early, hit hard” (Ehrlich, 1913). This empirical-based protocol may suggest, *a priori*, a rapid and complete eradication of the infection, but actually there are situations in which this approach is not the most appropriate, particularly nowadays, since many infections are caused by pathogens with pre-acquired resistance to some drugs. A more rational protocol may be based on PK/PD (pharmacokinetics/pharmacodynamics) indices, as the MIC (minimal inhibitory concentration) of an antibiotic, but several important factors are omitted in these indices (Udekwa and Levin, 2012). This work is focused on PK/PD models (with population dynamics included) as an alternative to empirical and indices-based protocols, since they can potentially

accommodate all aspects presented in Fig. 1, being a holistic (also complex and sometimes difficult) approach to the problem.

There are several models devoted to the study of pharmacokinetics (e.g., Nielsen et al., 2011) and disturbance (e.g., Mayer et al., 1995) variables. There are also specific models that give a mathematical form to the relationship between the killing agent and the infectious agent (pharmacodynamics variables), from an *in vitro* perspective (Iranzo et al., 2011; Landersdorfer et al., 2013; Pena-Miller et al., 2012, 2013; Torella et al., 2010; Wang et al., 2003; Wood et al., 2012). However, there are few holistic pharmacokinetic/pharmacodynamics (PK/PD) models and the number is even smaller if one consider only those models on multi-drug strategies. Lipsitch and Levin (1997) and Ankomah et al. (2013) explored such a model, based on the previous work of Levin and Udekwa (2010).

In this review, basic concepts concerning drug interactions are firstly presented. Secondly, five recent models were selected to illustrate the process depicted in Fig. 1, although most of them

were based on *in vitro* systems. Lastly, a brief reference is made to some variables (B1–3, Fig. 1) that were not extensively explored in the reviewed models, but that also deserves attention. The problem won't be analyzed on an epidemiologic basis, as done by other authors (Beardmore and Pena-Miller, 2010; Brown and Nathwani, 2005; Spicknall et al., 2013). However, it is recognized that infections are transmissible diseases and a really holistic approach should also take into account epidemiologic considerations. Instead, the system here is a single individual who has an infection to be treated and the problem to be addressed is: how to accomplish that effectively and efficiently?

2. Interaction between drugs

Before beginning to describe and analyze mathematical models, some basic concepts are introduced in this section in order to simplify the understanding of subsequent subjects. In the mathematical analysis of drug interactions two tools are usually needed: (i) a model that relates the dose to the effect (pharmacodynamics) and (ii) a criterion to identify the type of interaction between drugs.

2.1. Dose–effect models

The Hill equation (Eq. (1)) is widely used to relate a given dose with the respective effect (for concentration-dependent drugs; there are also approaches to handle time-dependent drugs), since it fits very well different situations.

$$E = \frac{E_{\max} D^n}{K^n + D^n} \quad (1)$$

In this equation, E is the effect caused by dose D and n (sigmoidicity factor), K (drug affinity) and $E_{\max}$ (maximum intensity effect) are model parameters. The graphical representation of Eq. (1) is a sigmoid, a curve with a saturation effect. Goutelle et al. (2008) described the origin and some applications of Hill equation.

Chou (2006) has demonstrated that Hill equation (among others, including the Michaelis–Menten equation for enzymes) may be derived from the median–effect equation (considered by the author as a universal equation to express dose–effect relationships), Eq. (2), which is based on the mass-action law.

$$\frac{f_a}{1-f_a} = \left(\frac{D}{D_{50}} \right)^m \quad (2)$$

In Eq. (2), f_a represents the fraction of inhibited cells (between 0 and 1) in the total population, D is the drug dose, D_{50} is the dose that inhibits 50% of the total population and m is a parameter that determines the shape of the dose–effect curve.

For antibiotics, which can have enzymes as targets, it is common to use dose–effect equations based on the inhibition mechanism of a specific enzyme. For instance, Pena-Miller et al. (2012) used an equation, Eq. (3), based on the competitive inhibition of two antibiotics on RNA polymerase.

$$E = \frac{1}{1 + D_A \sigma_A + D_B \sigma_B + D_A D_B \sigma_{AB}} \quad (3)$$

In Eq. (3), E represents the normalized inhibitory effect (the ratio between the reaction rate in the presence of drug and the reaction rate without drug), D_A and D_B correspond to the doses of each drug and σ_A , σ_B , σ_{AB} are model parameters.

The three equations above are only illustrative examples of dose–effect models. Jonker et al. (2005) reviewed this subject in detail, showing specific examples. Note that, when in the dose–effect equation the effect is not the variable that is directly measured, it is also required a conversion factor. For instance, if the optical density of a bacterial culture is assessed, and a dose–effect equation representing the inhibition of the drug on a specific

enzyme is used, it will be necessary to apply a conversion factor to account for all aspects not related to the enzyme, but that contributed to the measured growth.

2.2. Criteria for the classification of interactions between drugs

From a qualitative perspective, one may identify five types of drug interactions: additivity, synergy, antagonism, inertism and coalism (Fig. 2). The classification of the type of interaction is based on a comparison between individual and combined drug effects. Inertism and coalism are possible when, at least, one of the drugs has no individual effect. Additivity, synergy and antagonism are the most frequently explored drugs interactions in the literature. These drug interactions may be explained through the following analogy. Consider a pair of children, where one child reads a text to the other with a given loudness (sound intensity) that is constant. In another room there is a pair of adults in the same situation (same loudness, just to simplify the example, because this is not strictly necessary), but the text is different. When the pair of children and adults are joined in a small room and each continue to read the respective text, three situations may occur: (i) each narrator maintain the same loudness as in the individual rooms and therefore the sound intensity is twice that previously registered in each room (additivity); (ii) the narrators decrease the sound intensity in order to not disturb each other, being the sound intensity lower than twice that previously registered in each room (antagonism); (iii) the narrators increase both the sound intensity in an attempt to be heard better and the sound intensity in this situation is more than twice that previously registered in each room (synergy). In the situation described, loudness is the measured effect, the pair of children and adults represents two different drugs and the number of each pair in the room is equivalent to the dose. Although it was assigned the same attitude to both narrators when they are together in the same room, this is not strictly necessary to keep the validity of the concept. Note that even in this simple qualitative example a mathematical operation was applied: sum of sound intensities, which illustrates the difficulty to define drugs interactions without introducing quantitative criteria. But, now suppose the following: if the dose–effect relationship is not linear, which is common for drugs, the loudness of two pair of children is not twice that of one pair of children. Thus, when increasing the number of children and adults in the room, one cannot simply multiply the basal loudness by the number of elements, since erroneous conclusions would result. This point is focused, among others, by Lambert et al. (2003) and just shows an error that could be easily committed at this level.

Despite the classification of drug interactions may look like simple at a semantic level, this is not true when one analyzes them from a quantitative perspective. Indeed one may simply say that synergy occurs when a pair of drugs is more potent than the expected from the individual drugs. However, how does one obtain the expected effect? It is precisely at this point that scientific opinions diverge, existing more than one criterion. The two main quantitative criteria are Loewe additivity (Loewe, 1953) and Bliss independence (Bliss, 1939). Greco et al. (1995), in an extensive review, exploited the characteristics, advantages and limitations of both criteria, among others.

Loewe additivity is based on the purpose that a given drug cannot synergize with itself (Eq. (4)).

$$1 = \sum_{i=1}^n \frac{D_{i,mix}}{D_{i,E}} \quad (4)$$

$D_{i,E}$ represents the dose of drug i which has an effect E when administered individually, while on numerator, $D_{i,mix}$ corresponds to the dose of drug i that causes the same effect when administered

Fig. 2. Classification of drugs interactions (E – drug effect; E^{meas} – measured effect; E^{crt} – effect predicted by a given criteria; in the inertism case: $xy = CE$ or $xy = ED$). The interaction is classified after the comparison between the measured effect of the combined drugs (E^{meas}) and the one predicted by a given criteria from the measured effect of individual drugs (E^{crt}). The classification is depicted for dual combinations, although it may be extended to combinations involving more than two drugs. The scheme is based on the classification of Greco et al. (1995).

together with $(n-1)$ drugs. When Eq. (4) holds true, drugs are simply additive, namely the effect caused by the dose D of a drug is always the same, not changing in the presence of other drugs. When the right-hand side of Eq. (4) is less than 1, there is evidence of synergy, since the same effect is achieved with a lower dose of drugs, compared with the situation in which they are applied individually. When the right-hand side of Eq. (4) is higher than 1, the relationship between drugs is antagonistic. Eq. (4) is shown in its classical form, highlighting the dose variation for a constant effect, but synergy, additivity and antagonism concepts may be also interpreted in terms of an effect variation for a fixed dose.

The graphical aspect of Loewe criterion for a mixture of two drugs is an isobologram (Fig. 3). The isobologram or constant effect plot is a plane ($z = \text{constant}$) of a three-dimensional plot where the effect is represented in the z -axis and the concentration of each drug is represented on the other two axis, in a binary mixture. On isobolograms, synergy is represented by concave lines, antagonism corresponds to convex lines and additivity occurs when the isobologram is a straight line.

The Bliss independence criterion is based on the occurrence probability of n independent events, that is, events that can

Fig. 3. Isobolograms with cases of additivity, synergy and antagonism between drugs 1 and 2 (drug doses are represented in the normalized form, according to Eq. (4), for $n=2$).

exist simultaneously without changing each other probability (Eq. (5)).

$$E_{1,\dots,n} = \prod_{i=1}^n E_i \quad (5)$$

Bliss independence between drugs is verified when the measured effect of these drugs in combination ($E_{1,\dots,n}$) is equal to the value from Eq. (5), based on the individual effect of each drug (E_i). Synergistic and antagonistic interactions correspond to situations in which the measured effect of combined drugs is higher or lower, respectively, than the value from Eq. (5). Unlike Loewe additivity criterion, Bliss criterion allows self-synergism, which is the major failure pointed to it (Greco et al., 1995). However, it should be noted that situations for which the two criteria disagree with each other are due to the specific dose–effect model that is considered (Greco et al., 1995). Another aspect to be mentioned in the comparison between both criteria is that in Loewe additivity criterion, dosage units do not need to be the same for both drugs, since normalized dosage values (dimensionless) are used. This allows, for instance, evaluating the combined effect of a chemical compound with a heating treatment.

From a mechanistic perspective, drug interactions exist both at pharmacokinetics and pharmacodynamics levels. In the first case, synergy occurs, for instance, when a drug increases the bioavailability of other drug (Jawetz and Gunnison, 1953), while in the second case synergy may be present when both drugs act on enzymes involved in parallel and convergent metabolic pathways (Yeh et al., 2009). Johnson et al. (2004) reviewed different mechanisms of synergy and antagonism in antifungal therapies.

Although dose–effect models and criteria for the classification of drugs interactions have been presented separately, mathematical models may incorporate both aspects into one unique expression, as the $E_{\max}$ model proposed by Greco et al. (1995) (note that Eq. (3) is also in a form that incorporates drugs synergy in a dose–effect equation). These expressions may also be used to detect the type of drug interactions through a fitting procedure (Jonker et al., 2005; Greco et al., 1995). Thus, dose–effect models are usually in a form that includes the interaction between drugs, since there is one (or several) interaction factor that corrects the dose for a given effect (Bhagunde et al., 2012; Torella et al., 2010) or the effect for a given dose (Greco et al., 1995). Lee et al. (2007) presented some of these dose–effect models.

3. Modeling multi-drug therapies

The models proposed by Torella et al. (2010), Pena-Miller et al. (2012, 2013), Ankomah et al. (2013) and Wood et al. (2012), which are focused on the elimination of pathogens by the use of combined drugs, are explored in this section. These models were selected based on its recent publication date and also to give different perspectives/elements of the same problem. The common denominator between the first three models is: resistance development, drugs interaction and cellular competition, from an *in vitro* perspective. The fourth model is more complex, since it takes into account more PK/PD elements. The last model is focused on the prediction of the effect of multiple combined drugs. Whenever relevant, the models are accompanied by the respective set of equations that describe them, according to the information presented by its authors, since it seems important to illustrate the mathematical form of biological concepts.

3.1. Torella et al. (2010) model

The rationale behind the model suggested by Torella et al. (2010) is as follows: synergistic drugs reduce the clearance time

of the infection because they are more effective in combination than alone. However, they simultaneously increase the competitive advantage of resistant cells, when compared to susceptible ones, since these cells not only acquire resistance to one drug, as they are also less affected by the other drug. Conversely, antagonistic drugs have an opposite effect: they increase the clearance time, but reduce the competitive advantage of resistant cells. These experimentally validated results (Chait et al., 2007; Michel et al., 2008) were numerically achieved through the model.

This model is based on a set of differential equations (Eqs. (6)–(8)) obtained from the mass-action law applied to the different cell phenotypes that we may find in an infection.

$$\frac{d}{dt}X_s = X_s[G - K(D_s)] - 2\mu GX_s \quad (6)$$

$$\frac{d}{dt}X_r = X_r[G - K(D_r)] + 2\mu GX_s - \mu GX_r \quad (7)$$

$$\frac{d}{dt}X_{mr} = \mu GX_r \quad (8)$$

According to Eqs. (6)–(8), the susceptible cells (X_s) may be converted to resistant cells to one drug (X_r) by mutation, which in turn may mutate to a multi-drug resistant phenotype (X_{mr}). These X_{mr} cells cannot be eliminated by any of the two drugs in use, but, also, they are not allowed to grow. The evolution of the number of cells of each phenotype along the incubation time depends on the growth rate (G), on the death rate due to antibiotic action (K), and on the mutation frequency (μ). The growth and death rates depend in turn on the total ($X_s + X_r + X_{mr}$) and maximum ($X_{\max}$) cells number, on the drugs doses (D) and on the interaction parameter between the drugs ($\varepsilon > 0$ for synergy; $\varepsilon = 0$ for additivity; $\varepsilon < 0$ for antagonism), as shown by Eqs. (9)–(12).

$$G = g \left(1 - \frac{X_s + X_r + X_{mr}}{X_{\max}} \right) \quad (9)$$

$$K(D_y) = \frac{k D_y}{1 + D_y} \quad (10)$$

$$D_s = D(2 + \varepsilon) \quad (11)$$

$$D_r = D \quad (12)$$

In Eqs. (9) and (10), variables g and k correspond to the maximum cell growth rate and killing rate, respectively, while D_y is the effective drug dose which acted on the microbial phenotype y (it is the drug concentration corresponding to the perceived effect, which may be higher or lower than the physical concentration depending on the existence of drugs interactions). Only the cell death promoted by the action of drugs was considered and both drugs are assumed to be administered at the same dose. The resistance developed by cells has no fitness costs according to the model (which is not always true, e.g., Maclean et al., 2010) and only causes a reduction in the drug concentration that reacts with the microorganisms (D_y) (Eqs. (11) and (12)). In this model, the dose–effect relationship is represented by a Hill equation (Eq. (10)) and a logistic growth is assumed by setting a maximum population sustainable by the system (Eq. (9)), which allows to control the degree of competition between cells through the value of $X_{\max}$.

The authors of the model evaluated the infection therapy based on two criteria: (i) the inverse of the clearance time (t_{clear}^{-1}) and (ii) the generation of multi-drug resistant cells, quantified by the inverse of the number of multi-drug resistant cells (X_{mr}^{-1}).

The system of differential equations was solved starting from an initial population composed of susceptible and resistant cells (these last in a quantity proportional to the mutation rate). The results demonstrated the existence of two different scenarios, depending on the competition level between cells (Fig. 4).

Fig. 4. Effect of the drug interaction parameter (ε) in preventing the emergence of multi-drug resistant cells (X_{mr}^{-1} , black line) and on the treatment efficiency (t_{clear}^{-1} , grey line) (A) with and (B) without competition. Adapted from [Torella et al. \(2010\)](#).

With competition (Fig. 4A), the model shows that there is a compromise between the clearance time and the prevention of multi-drug resistance: the two goals evolve inversely as a function of the ε parameter. However, there is a threshold level of interaction (ε^*), from which clearance time stabilizes and, from that point, an increase in the synergy between drugs only enhances multi-drug resistance. Under these conditions, synergistic drugs are favorable to the development of multi-drug resistance because susceptible cells are more easily killed, leaving an opportunity window (nutrients and space) to the development of resistant and multi-drug resistant cells – competition is relaxed. The existence of a minimum clearance time (the plateau in Fig. 4A) is due to the fact that the killing rate of resistant cells (the last to die when $\varepsilon > \varepsilon^*$) does not depend on the level of interaction between drugs (Eqs. (8) and (12)), since one drug has no effect on these cells and can no longer enhance the effect of the other. It should be stated that the threshold level of drug interaction (ε^*) is not always located in a synergy zone ($\varepsilon > 0$). Effectively, this threshold depends on several factors, being the most important the resistance acquisition rate: for high rates, the ε^* value is located in an antagonism zone ($\varepsilon < 0$). In these

cases, the use of synergistic drugs should be avoided because it is not advantageous.

On the other hand, when local conditions are not limiting (Fig. 4B) – abundance of space and nutrients, arising from $(X_s + X_r + X_{mr}) \ll X_{max}$ in Eq. (9) – the increase in the degree of synergy is always favorable as it continuously reduces the probability of multi-drug resistance, although t_{clear}^{-1} keeps the same asymptotic growth profile. According to the authors of the model, the difference observed in the profile of X_{mr}^{-1} between both competition situations (Fig. 4A and B) derives from the fact that in non-limiting conditions synergy promotes the rapid removal of susceptible cells, which are in the hierarchical basis of the development of multi-drug resistant cells. This phenomenon is also present in limiting conditions, but the relaxation of competition due to the death of susceptible cells assumes a greater importance in these conditions (it is a balance between the relative effects of X_s and X_{tot} in X_{mr} ; Eq. (8)).

Therefore, the model developed by Torella and colleagues shows that the use of synergistic drugs (commonly associated to the “hit hard” policy) may not be advantageous in all infections cases, particularly those in which competition between cells or mutation frequency is high. However, it should be stated that the interaction factor ε may be a function of the drug dose ([Ankomah and Levin, 2012](#)). In addition, it is possible the existence of mutations that simultaneously confer resistance to both drugs and the rate of resistance development changes with the type and the intensity of interaction between drugs ([Michel et al., 2008](#)). Moreover, the model considers the infection cleared even if there are multi-drug resistant cells alive, which is questionable. Multi-drug resistant cells can grow even in a small number, especially if these cells arise in a huge number while the immune system is occupied in fighting the other phenotypes (possibly in another compartment). This phenotype would assume more importance in the model if it was allowed to grow. This aspect does not affect the reliability of the results obtained, although it may be important in living organisms. The same may be pointed to the threshold bacteria number under which the infection was considered cleared (the value will depend on several factors, as the immunological state of the patient).

3.2. [Pena-Miller et al. \(2013\)](#) model

[Pena-Miller et al. \(2013\)](#) have proposed and performed the experimental validation of a theoretical model that demonstrates the role of synergistic drugs in the selection of resistant bacteria and on the control of an infection. The novelty of this work consisted in the addition of a time component to the concept of synergy.

The model developed by [Pena-Miller et al. \(2013\)](#) is based on the logistic growth of a bacterial population that is inhibited by a pair of synergistic drugs, against which bacteria develop resistance:

$$\frac{d}{dt}X_s = X_s \left[1 - \frac{(X_s + X_{mr})}{X_{max}} \right] g - X_s(k(\theta) + \mu) \quad (13)$$

$$\frac{d}{dt}X_{mr} = X_{mr} \left[1 - \frac{(X_s + X_{mr})}{X_{max}} \right] g + \mu X_s \quad (14)$$

$$k(\theta) = 0.1 + \lambda\theta(1 - \theta) \quad (15)$$

In Eqs. (13)–(15), X_s and X_{mr} represent the susceptible and multi-drug resistant bacteria, respectively, μ is the mutation rate, g is the maximum growth rate, θ is the dose fraction of one of the drugs in the binary mixture, k represents the cell death rate caused by antibiotics (being a function of θ) and λ is a factor that modifies the synergy intensity between drugs. The numerical simulation of Eqs. (13)–(15) has shown that the dose with the highest synergy at the beginning of the treatment presents the highest degree of antagonism after some time, depending on the value of λ (Fig. 5).

Fig. 5. Total cells fraction $((X_s + X_{mr})/X_{\max})$ as a function of the drug fraction, θ , for (A) $\lambda = 1$ and (B) $\lambda = 0.5$. Each line is relative to a given elapsed time from the beginning of the treatment (the initial population was set to $X_{s0} = 0.1X_{\max}$ and $X_{mr0} = 0$). The time increase is represented by an increase in the lines width and brightness.

Adapted from [Pena-Miller et al. \(2013\)](#).

In [Fig. 5](#), drugs exhibit synergy while the curves are concave (any drug combination is able to inhibit the growth more intensely than individual drugs, at the edges of the x -axis), a situation that is verified in the early stage of the treatment. However, synergy interaction evolves to antagonism over time. Based on Eqs. (13)–(15), it is possible to conclude that this is the result of susceptible bacteria elimination by drugs, which leaves an opportunity window to the development of multi-drug resistant bacteria. In fact, logistic growth is assumed. Thus, the faster the elimination of susceptible bacteria, the easier will be the prevalence of multi-drug resistant bacteria. In this way, since synergistic drug combinations eliminate more rapidly the susceptible bacteria, they automatically potentiate the growth of the multi-drug resistant population. Consequently, synergistic drugs are selective for the multi-drug resistant phenotype and therefore become ineffective over time, showing poorer results than a monotherapy (edges of x -axis in [Fig. 5](#)). Although the authors did not refer that, it would be expected exactly the inverse situation for an antagonistic pair of drugs: the 0.5:0.5 drug combination would become the best combination (evolve to synergism) along time. The increase of λ anticipates the moment at which combined drugs become less effective than individual drugs (concavity inversion) ([Fig. 5](#)). For an infinite time, the total cellular fraction profiles evolve to the horizontal asymptote

with the value of 1 or 100% and this population is exclusively composed of multi-drug resistant cells.

The model allows the development of a multi-drug resistant population provided that at least one single multi-drug resistant cell appears in the system by mutation of one susceptible cell (Eq. (14)), assuming that the initial population is composed of only susceptible cells.

The authors have also demonstrated that the expression of drug resistance genes, such as those that codify for efflux pumps, is enhanced by the use of combined drugs. The knockout of these resistance genes deleted the concavity inversion shown in [Fig. 5](#). That is, initial synergistic combinations were able to maintain the synergy over time. These tests have nearly (not exactly) the same effect of imposing $\mu = 0$ in Eqs. (13)–(15), i.e., the formation of drug-resistant mutants from susceptible bacteria was switched off. This can be accomplished on experiments through the use of a third drug that suppresses the effect of resistance genes, when possible.

It should be noted that some features in [Fig. 5](#) are parameter-dependent. Indeed, if a more potent pair of drugs is considered, that is, if a higher value is assigned to the first term in the right-hand side of Eq. (15) (for instance 1, instead of 0.1), it is possible to observe three main changes. Firstly, the concavity inversion occurs earlier, as seen with parameter λ . The curves are initially concave because the evolution profile of susceptible cells is concave (maximal killing occurs for the more synergistic combination – 0.5:0.5) and these cells are more numerous than multi-drug resistant cells, whose evolution profile is convex (since the opportunity window is maximal at a combination of 0.5:0.5). As the time increases, multi-drug resistant cells grow while susceptible cells die, and when multi-drug resistant cells are in more significant number than the susceptible ones, the concavity inverts. Since more potent drugs kill faster the susceptible bacteria, they also induce an earliest concavity inversion. The second observation is that the curves obtained have not the same profile as that in [Fig. 5](#), since they are simple parabolic lines. This can be explained by the fact that the evolution profile of each population is more flat. Lastly, under these conditions, the total cellular fraction initially decreases because the reduction in the number of susceptible cells is not surpassed by the increase of the multi-drug resistant population, due to the high effect of drugs. This was not observed in [Fig. 5](#), because the weak effect of drugs allows initially an increase in the population of susceptible cells (growth rate is higher than killing rate), that offsets the little increase in the population of multi-drug resistant cells, at the same time. It is also important to see that the increase in the value of λ corresponds to an increase in the synergy and strength of the drug pairs. However, the increase λ is not as significant as changing the first right-hand side term of Eq. (15), since λ is multiplied by a factor that ranges between 0 and 0.25.

Thus, it can be remarked that the work of [Pena-Miller et al. \(2013\)](#) complements some conclusions of the previous model ([Torella et al., 2010](#)). In fact, synergy is not always the better choice. Particularly, synergistic drugs should only be used when the mutation rate is low and/or to quickly eliminate infections, since for prolonged treatment times, the use of synergistic combinations becomes ineffective due to the selection of drug-resistant phenotypes. The cell competition (logistic growth) considered in both models was again a key feature for these conclusions, since it acted as the selection factor.

3.3. [Pena-Miller et al. \(2012\)](#) model

The model proposed by [Pena-Miller et al. \(2012\)](#) aims to determine the best administration algorithm of two synergistic drugs in order to eliminate a population of pathogenic bacteria (both drug susceptible and resistant strains) without harming the host's flora

(commensal bacteria), which can be a common side effect in the treatment of infections (Dethlefsen et al., 2008).

The authors based their model on a chemostat in which pathogenic and commensal bacteria grown together. The reactor received an adjustable flow of drug A (at a dilution rate $\alpha(t)$), at a concentration D_{A0} and an adjustable flow rate of drug B (at a dilution rate $\beta(t)$), at a concentration D_{B0} . Both drugs act independently and synergistically in the same enzyme. The flow rate obtained by mixing both drugs has a constant substrate concentration S_0 and the chemostat has a constant dilution rate: $\delta = \alpha(t) + \beta(t)$. The set of differential equations arising from the application of the mass-action law is as follows:

$$\frac{d}{dt}S = \delta(S_0 - S) - \langle r_c(S), C \rangle - \langle r_p(S), P \rangle \quad (16)$$

$$\frac{d}{dt}C = \mu_c[G_c(S, D_A, D_B)C] - \delta C \quad (17)$$

$$\frac{d}{dt}P = \mu_p[G_p(S, D_A, D_B)P] - \delta P \quad (18)$$

$$\frac{d}{dt}D_A = \alpha(t)D_{A0} - \delta D_A - aD_A(\vec{1}, C) + (\vec{1}, P) \quad (19)$$

$$\frac{d}{dt}D_B = \beta(t)D_{B0} - \delta D_B - bD_B(\vec{1}, C) + (\vec{1}, P) \quad (20)$$

where the growth rate of the cell phenotype x is given by the following expression:

$$G_x(S, D_A, D_B) = c \left(\frac{Sv_{\max}^x}{K + S} \right) \left(1 - \frac{k_{1A}^x D_A}{k_{2A}^x + D_A} \right) \left(1 - \frac{k_{1B}^x D_B}{k_{2B}^x + D_B} \right) \quad (21)$$

The authors used a matrix notation to broaden the scope of the model to the various phenotypes that may be present in the infection (drug susceptible, drug resistant and multi-drug resistant). P and C represent vectors wherein each element corresponds to the pathogenic and commensal biomass concentration, respectively, concerning a given phenotype. The brackets $\langle \cdot \rangle$ and $\langle \cdot \rangle$ represent the inner product of the vectors in their inside. The vectors r_c and r_p contain the substrate (S) uptake rate for the different bacterial phenotypes and its expression (Michaelis–Menten kinetics) corresponds to the first bracketed element on the right-hand side of Eq. (21) ($v_{\max}$ is the maximum substrate uptake rate and K is the substrate affinity parameter). Matrices μ_c and μ_p are respective to the mutation rate of different cell phenotypes (as only one commensal phenotype is present and, by imposition of the authors, it cannot develop resistance, $\mu_c = 1$). The constants a and b reflect the binding affinity of drugs to the cells and k constants are model parameters derived by the authors from an enzymatic inhibition mechanism. The c parameter is a conversion factor between the consumed substrate and the biomass that is formed and the vector $\vec{1}$ is composed of n or m 1's elements, where n and m are the number of pathogenic and commensal bacteria phenotypes, respectively.

With the system of Eqs. (16)–(21), the authors aimed to find which functions $\alpha(t)$ and $\beta(t)$ were able to maximize the difference between the number of commensal and pathogenic bacteria over a treatment with a time extension T (an optimization problem, by its nature):

$$\max[J(\alpha(t), \beta(t))] = \max \left[\int_0^T (\vec{1}, C) - (\vec{1}, P) dt \right] \quad (22)$$

The results suggested that the best administration algorithm consists in a sequential and alternating (cycling) administration of each drug and does not depend on the level of interaction between drugs. This administration strategy allows the growth of commensals over pathogenic bacteria, even if commensals have a lower

Fig. 6. Evolution of the population of pathogenic (both susceptible and drug resistant) and commensal bacteria through two algorithms of drugs administration: (A) applying both drugs simultaneously, and (B) applying the two drugs sequentially and alternating (each bright and dark segment represents one of the two drugs). At time $t = 0$ h (beginning of the treatment) it is assumed that pathogens had time enough to dominate over commensals.

Adapted from Pena-Miller et al. (2012).

competitive advantage than pathogenic bacteria (Fig. 6). The physical system selected for this model – a chemostat – had a major role in the results, since it allowed the continuous removal of cells, which may exceed the cells generation rate and lead to a net reduction in the bacteria population. This is one of the reasons that explains the infection clearance, though the use of a drug combination to which pathogens are resistant. In a living system, this continuous clearance factor should be carefully applied (the immune system, for instance, may contribute to this role, but maybe not with the performance considered in the model, that will also depend on the infection location and its physical structure).

It should be noted that, in this model, the multi-drug resistant phenotype is also sensible to the drugs (though, at a less extent than susceptible ones). Otherwise, this phenotype would become dominant, since the natural senescence of cells was not considered, and the profiles seen in Fig. 6 would be different. The definition of multi-drug resistance was, for instance, different from what it was seen for the model of Torella et al. (2010), which further raises the question of how to mathematically translate this biological concept?

One principal conclusion that arises from the model is that the administration strategy of the antibiotics may be even more important than the type of antibiotics that are used. However, although the cycling strategy can be easily implementable computationally

(by negative feedback, switching the drug when the phenotypes resistant to this drug begin to be more numerous than the other phenotypes present), in practice it can be difficult to follow the evolution of each bacterial population in real time. Nevertheless, this model takes into account an aspect not directly considered in other works: the importance of commensal bacteria in the treatment and *vice versa* (since they are one more competitive element). The role of commensal bacteria in preventing multi-drug resistant infections was considered in some cases even more important than resistance transmission between pathogens (Tosh and McDonald, 2012).

3.4. Ankomah et al. (2013) model

The work of Ankomah et al. (2013) was based on the model of Levin and Udekwe (2010), which includes both pharmacodynamics and pharmacokinetics considerations. In comparison to the previously described models, this one introduces additional variables that more closely approach it to infections in living organisms.

Firstly, there is a differentiation between actively growing and persister cells. Persister cells are genetically susceptible to the drugs, but they are phenotypically resistant to them. This is achieved through a lower growth rate and the model allows the conversion of actively growing cells into persisters and *vice versa*. For both types of cells there are three different genotypes: resistant to a drug A, resistant to drug B and multi-drug resistant. Additionally, the authors took into consideration the existence of cell “sanctuaries” – regions in the infected area where the concentration of antibiotics is especially low, as biofilms. Both persister cells and sanctuaries act (although differently) as a source to replenish actively growing cells killed by antibiotics.

Secondly, the effect of the antibiotics (modeled by a Hill equation) is dependent on the total concentration of cells and on the cells growth rate, which depends on the limiting resource concentration, following a Monod kinetics. From a pharmacokinetics perspective, it was considered a periodic administration of the antibiotics, as well as an exponential decay of the respective concentration.

Since the authors used a chemostat as a physical model, the resulting equations (and respective limitations) are very similar to the set of differential equations seen for the Pena-Miller et al. (2012) model, which stem from a mass balance (they are not presented for conciseness).

The model simulations performed by the authors demonstrated that multi-drug resistant cells only arise when there is enough time to the development of susceptible and single-drug resistant cells, which is the case when only one drug is administered. Persister cells increase the clearance time of the infection, but do not necessarily compromise the cure. On the other hand, the cure may be compromised by the existence of cell sanctuaries.

Contrarily to what it was seen for Torella et al. (2010) and Pena-Miller et al. (2013) models, in this model synergistic combinations were not outperformed by antagonistic combinations, which may be explained by several factors, as the use of different parameters values or the consideration of a growth kinetics not following a logistic equation (although competition was present in the availability of a limiting resource). This further illustrates one weakness of modeling: the behavioral dependence on the selected parameters (and the difficulty to obtain reliable estimates for those parameters).

3.5. Wood et al. (2012) model

The model of Wood et al. (2012) may seem unframed from the previous models, since its only goal is to predict the killing efficacy of a drug combination (no pharmacokinetics variables were considered, since it was intended to *in vitro* systems). Indeed, it

represents a different approach to what it was seen previously, because individual pharmacodynamics events are not considered in the resulting model. The overall effect of a drug combination is simply predicted from the pairwise and individual effects, in a mechanism-independent manner.

This model is based on probabilistic arguments. The equation that allows predicting the combined effect of multiple drugs is a multi-variable probability distribution obtained by the minimization of an error function, in this case an entropy function (which has degrees of freedom that measure drug interactions). The (unique) expression obtained in this way overlaps Isserlis theorem (Michalowicz et al., 2011), arising in an algebraic form rather simple when compared with systems of differential equations. For instance, for a combination of three drugs (A, B, C), the effect may be predicted through Eq. (23).

$$E_{A,B,C} = E_A E_{B,C} + E_B E_{A,C} + E_C E_{A,B} - 2E_A E_B E_C \quad (23)$$

In Eq. (23), E_i is the effect measured in the presence of drug i (e.g., the microbial growth rate). The structure of this model, as well as the experimental validation performed, with *Escherichia coli* and *Staphylococcus aureus*, suggests that a probabilistic manipulation of the effect variable is sufficient for a good prediction and does not depend on the knowledge of the molecular action of the drug. However, tests with individual drugs and with drugs combined in pairs are required as inputs for this model, for each microbial species (including mutants of the same specie) and for different dosages (the effect is dose-dependent and the model has only been validated for a limited range of drug concentrations).

Since higher order effects can be expressed in terms of lower order effects (individual and pairs effects), this means that no new information is added to lower order effects. Thus, according to the model, the effect of N combined drugs is not the result of any new event, but only an intensity balance of lower order effects, which depends on the proportion of each drug present. The exception occurs when the drugs have no individual effect, but develop an effect when combined together (coalism), which, however, is not so frequent (Wood et al., 2012).

From a practical point of view, the possibility of using a mechanistic-free model is quite promising. However, can Eq. (23) predict the effect of N drugs on *in vivo* systems (or even for all *in vitro* drugs experiments: tube cultures, continuous devices, etc.)? Although experimental data is currently insufficient to answer this question, it is expected that Eq. (23) could be useful in finding new drug combinations, since it theoretically reduces the set of required experimental tests. On the other hand, it remains to be explored the possibility of combining Eq. (23) with dose–effect models, for a wide range of doses. This eventual association may broaden the scope of the model to any dose without the need for testing each different dose, although it seems difficult to realize, since the interactions between drugs may change as a function of drugs concentration, in a manner that is not apparently logic, nor predictable from mechanistic arguments (Ankomah et al., 2013).

4. Pharmacokinetics, pharmacodynamics and disturbance variables

This section provides a brief discussion on disturbance (immune system and adherence to the therapy), pharmacokinetics and pharmacodynamics (biofilm formation, single/double mutations and horizontal gene transfer, age and composition of the initial population of pathogens) variables.

4.1. Immune system

The adaptive immune system is responsible for the development of defense mechanisms after pathogens cross the barriers

imposed by the host's innate immune system. The action of the immune system during an infection aims to reduce the number of pathogens. If this response is selective for a given phenotype of the pathogenic population (as in *Cryptococcus neoformans* infections, where biofilms are more resistant than planktonic cells to the molecules produced in the immune response, [Martinez and Casadevall, 2006](#)), the intercellular competition may be modified, which would favor the most adapted cells. Thus, models should include an additional clearance factor developed by the host immune system ([Czock and Keller, 2007](#); [D'Agata et al., 2008](#)), as well as a threshold pathogen concentration (or killing rate) under which the immune response is self-sufficient. However, it should also be noted that the immune system has a random component in its response ([Mayer et al., 1995](#)).

4.2. Adherence to the therapy

The non-adherence of a patient to the therapy that was prescribed to him is quite common in specific infection types ([Kardas, 2002](#)). An important consequence of this comportment is the emergence of drug resistance ([Kardas, 2002](#)), since a selection window to resistant phenotypes may arise ([Yeh et al., 2009](#)), due to the drug concentration profile that is developed.

Through the simulation of a mathematical model relative to the non-adherence, [Ankomah and Levin \(2012\)](#) have precisely observed the resistance development to antibiotics under different scenarios of non-adherence. Although this phenomenon may be modeled, the non-adherence is hardly predictable, since it depends on the attitude of each patient.

4.3. Pharmacokinetics

The use of any drug in the human body follows the well-known ADMET rule (administration, distribution, metabolism, elimination, toxicity) ([van de Waterbeemd and Gifford, 2003](#)). After the intake, the drug crosses the biological barriers, dilutes in the local fluid and reaches the target sites in the organism. The step that follows distribution consists in the interaction of the drug with the various organs that it reaches, depending on the type of drug, route of administration and its specificity. At this stage the drug not only interacts with the intended targets, but also with the organic systems that are responsible for the drug metabolism, such as the liver if the drug flows into the blood. Finally, the drug is eliminated from the body by different ways. The remaining amount can have desirable and/or toxic effects. From this cycle, the effect with more interest to this review is the change of drug concentration, both over time and space (multi-compartment models may be useful at this respect).

The concentration profile depends on the drug class, the frequency of administration and the rate of drug elimination. As the drug elimination occurs continuously, contrarily to the drug intake, the concentration profile is a decreasing curve between drug intake moments, being also periodic for a periodic administration (common with antibiotics). In a model, this is equivalent to an additional clearance factor for the drug concentration ([Ankomah et al., 2013](#)). Therefore, pharmacokinetics has effects on the development of resistance to drugs – selection window ([Michel et al., 2008](#)) – as well as on the minimal inhibitory concentration (MIC) of certain drugs, whose value depends on the concentration of other(s) synergistic(s) drug(s) administered ([Bhagunde et al., 2012](#)).

4.4. Biofilm formation

Microbial cells may exist in a planktonic state or adhered to substrates forming cell aggregates named biofilms. It is well-known that biofilm formation confers microbial resistance to antimicrobial

compounds ([Anderson and O'Toole, 2008](#); [Grobe et al., 2002](#); [Høiby et al., 2010](#)).

The inclusion of biofilms formation in models may influence various parameters. Depending on the level of differentiation of the biofilm, its compaction and composition, mass transfer resistance assumes a more or less important role. In the presence of mass transfer resistance, the diffusion of species (including substrate, drugs, living/dead cells) to/from the biofilm can be slower ([Lynch et al., 2007](#)). Thus, all parameters that are concentration-dependent, like growth rate and death rate are affected by biofilm formation. Furthermore, the interaction between drugs may be affected if, for instance, there is a differential diffusion of one drug in comparison to the other.

Biofilms may also be considered as micro-compartment where chemical gradients exist ([Stewart, 2003](#)). Therefore, considerations on the resistance development concerning heterogeneous environments ([Hermesen et al., 2012](#)) are also valid for biofilms. These considerations are related with the opportunity window (where resistant bacteria may arise due to competitive advantage) that exists for specific ranges of antibiotic concentrations ([Hermesen et al., 2012](#)).

In addition to phenotypic resistance, biofilms may also enhance drug resistance by inducing changes in the gene expression of microorganisms ([Zhang and Mah, 2008](#)).

It should be noted that there is a bilateral relationship between biofilm formation and cellular competition. The competition enhances biofilms formation and biofilms are a competitive advantage for cells. Since competition is very important in modeling the growth of microorganisms, as previously seen, thus, the formation of biofilms is also important from that perspective.

4.5. Single/double mutations and horizontal gene transfer

The selection of the adequate drugs is among the most important factors to be considered when planning a therapy to eradicate an infection. Effectively, a drug combination to which a pathogen is resistant will hardly clear the infection. However, in an attempt to simplify the resulting models, the authors often consider a limited number of mutations types that conduct to the resistance. According to [Maclean et al. \(2010\)](#), a given mutation can lead to the resistance to one or multiple drugs and each one may or may not have a fitness cost (e.g., growth rate reduction in the absence of the drug), which may be reversed in subsequent mutations. The fitness cost is one important selection factor in a given environment with/without drug (this cost was, for instance, considered in the model of [Pena-Miller et al., 2012](#)). In addition, resistance genes can be horizontally transferred between bacteria ([Barlow, 2009](#)), resulting in a resistance development rate higher than the single mutation rate.

4.6. Age and composition of the infectious population of microorganisms

The composition of the microbial population when the treatment is started depends on the elapsed time relative to the moment of the infection and on the conditions in the infected site, including cell competition and the action of the immune system. Since models based on the mass-action law require the definition of initial conditions for the resolution of a system of differential equations, it is important to make a good estimate of the composition of the initial infectious population. It may be recognized the importance of this estimate in real clinical situations, since the treatment protocol, as well as its results, depend clearly on the time period between the pathogen invasion and the beginning of the treatment ([Deresinski, 2007](#); [Houck et al., 2004](#)).

Fig. 7. Universal protocol for infections treatment through a multi-drug therapy, based on a mathematical model (optimization problem). This protocol requires standard and actualized information about common pathogens, namely dose–effect curves for several drugs. The standard information is modified and complemented with specific data about the case (individual-adapted therapy), as, for instance, pre-acquired resistance to antibiotics and the location of the infection. At this stage, technological advances are essential for diagnostic purposes, since they represent the source of the data. In addition, also epidemiologic data is required as input, because infections are transmissible diseases. All this information is automatically converted to numerical and logical data and processed in the model. The output of the model (optimal solution) is the best strategy to be implemented in order to satisfy the three goals set in Fig. 1: rapid recovery, avoid resistance emergence and minimize side effects. The output strategy would be a decision support to the medical team.

The variables of interest to define the composition of the initial microbial population are: (i) the type and number of pathogens, (ii) the fraction of susceptible microorganisms, (iii) the fraction of microorganisms resistant to each drug and (iv) the fraction that presents multiple resistance. This estimate is usually accomplished based on the mutation rate and on the population size at the onset of the treatment (Jumbe et al., 2003). However, the mutation rate is not always a constant and generalized value, because local conditions are diversified. For instance, in *Pseudomonas aeruginosa* respiratory infections, the transformation of the mucoid phenotype into the non-mucoid one is intrinsically linked to free radical formation by immune cells (Bjarnsholt et al., 2009). The composition may also be analyzed through distinguishing between fast-growing and persister (dormant) cells, as in Ankomah et al. (2013). Persister cells have enhanced resistance to drugs (Dawson et al., 2011; Keren et al., 2004; Lewis, 2010).

In addition to cellular competition, two other effects may be identified as being dependent on the total number of cells in the population. Firstly, it was seen that the MIC of an antibiotic may depend on the dimension of the population (Ankomah et al., 2013). Secondly, density-dependent mechanisms of resistance (where biofilm formation may be included) will depend on the total number of cells, at least for certain bacteria species, according to quorum-sensing effects (Defoirdt et al., 2010).

5. Concluding remarks

5.1. Current limitations

Although the models reviewed here seem quite simple, it should be stated that there are several other aspects to consider in the improvement of these models, which were not covered in this review. Some aspects are specific of a given type of infection; for example, intracellular invasion is frequent in tuberculosis or typhoid fever (Brown et al., 2006; Eswarappa, 2010). On the other hand, there are aspects such as bacterial migration (Hermesen et al., 2012), co-existence (Colijn et al., 2010) and host–pathogen equilibrium in persistent infections (Eswarappa, 2010) that are more frequent. Obviously, the more parameters and behaviors are

included, the more complex becomes the model. The relationship kept between drugs in multi-drug therapies is another complexity factor, as the interaction depends not only on the drugs concentration, but also on biological variables.

In the first four models that were presented, the therapies simulated were composed by only two drugs, which reflects a common practice in the literature on this research field (Wood et al., 2012). However, it would be challenging to increase the number of drugs or, more importantly, the number of drugs interactions (as in the model developed by Wood et al., 2012). For instance, consider a therapy where one drug is administered together with a synergistic drug and an antagonistic drug, either simultaneously or with alternation between them. Would it be possible to benefit from the different eradication velocity and competitive advantage characteristic of each drug interaction in such a case?

5.2. Future perspective

The review finishes the same way it started: drug resistance is the key feature of the problem under discussion. Indeed, all variables presented in Fig. 1 may be direct or indirectly linked to drug resistance.

The models reviewed here were not specifically proposed for the simulation of a drug therapy in a real living organism. Rather, they are relative to *in vitro* systems, where, some events that occur in living organisms were reproduced, to accomplish other purposes. Thus, it is not the intention of this study to criticize such models for the realism of its result. Instead, some clues are provided to help linking between *in vitro* results and *in vivo* ones, since the required tools seem to exist. Although *in vitro* models are useful, some results obtained by this way may not be valid in living organisms. It may be argued that mathematical models are just that and we cite Box and Draper (1987): “All models are wrong, some are useful”. However, we ask if these models could not be more than that (not just in a scientific context, but also from the clinical perspective)?

Mathematical models are successfully used in many areas as a prediction tool, such as in meteorology or in geology. Their use to predict the output of an (*in vivo*) infection treatment or, in a reverse sense, to define the best treatment strategy to achieve a

given set of goals, would be a significant advance in medicine. One main problem is the high complexity of the systems involved (both the host and pathogens), to which one may also add the standing evolution of pathogenic agents. A wind wave is always a movement of an air mass, either today or in the past, while biological systems have a pool of DNA that allows them to evolve. Furthermore, in Biology it is well known that there are always exceptions for a given rule. Nevertheless, we present in Fig. 7 a possible scheme of what would be an ideal and generalized flow sheet to be used in the rational design of a protocol. From a mathematical perspective, the central box in Fig. 7 represents an optimization problem with a multi-objective cost function (box C of Fig. 1), a set of constraints (boxes B of Fig. 1) and where several degrees of freedom (design variables) may be explored (box A of Fig. 1). While the cost function and design variables may be easily defined, the higher complexity still remains in defining the constraints. Apart from the problem definition, only an optimization algorithm is required to solve the problem, on a pure mathematical basis.

It is questionable if such a universal flow sheet would be feasible, as well as its utility (given the high complexity expected) and reliability. A database with *in vitro* dose–effect curves for different drugs combinations (with two or more drugs) and different pathogens could even be achieved, for example, by a model such as that of Wood et al. (2012). However, the constant evolution of microorganisms is not controllable and is hardly predictable, so that this database would quickly become outdated. Furthermore, how this *in vitro* data would be connected to the mathematical model based on living organisms is still a question. On the other hand, it is difficult to meet in a short time the required information about the host and the pathogen, which is one of the main reasons to still apply broad-spectrum drugs to treat common infection cases.

The current analysis has focused essentially on the treatment of bacterial infections, but some conclusions can be transposed, with the required care, to the treatment of other infections types, such as viral infections, and also to tumor therapies, as these are situations in which multi-drug therapies are also employed, with the (non-exclusive) goal of killing live entities.

Acknowledgments

This work was supported by the Operational Program for Competitiveness Factors – COMPETE and by Portuguese Foundation for Science and Technology through Project Phytodisinfectants – PTDC/DTP-SAP/1078/2012 (COMPETE: FCOMP-01-0124-FEDER-028765), the PhD grant awarded to Ana Abreu (SFRH/BD/84393/2012) and the Post-Doc grant awarded to Lúcia Simões (SFRH/BPD/81982/2011). Also, this work was undertaken as part of the European Research Project SusClean (Contract no. FP7-KBBE-2011-5, project number: 287514) and the COST Action FA1202. The authors are solely responsible for this work. It does not represent the opinion of the Community, and the Community is not responsible for any use that might be made of data appearing herein.

References

- Anderson, G.G., O'Toole, G.A., 2008. Innate and induced resistance mechanisms of bacterial biofilms. *Curr. Top. Microbiol. Immunol.* 322, 85–105.
- Ankomah, P., Johnson, P.J.T., Levin, B.R., 2013. The pharmacology, population and evolutionary dynamics of multi-drug therapy: experiments with *S. aureus* and *E. coli* and computer simulations. *PLoS Pathog.* 9, e1003300.
- Ankomah, P., Levin, B.R., 2012. Two-drug antimicrobial chemotherapy: a mathematical model and experiments with *Mycobacterium marinum*. *PLoS Pathog.* 8, e1002487.
- Barlow, M., 2009. What antimicrobial resistance has taught us about horizontal gene transfer? *Methods Mol. Biol.* 532, 397–411.
- Bartlett, J.G., Gilbert, D.N., Spellberg, B., 2013. Seven ways to preserve the miracle of antibiotics. *Clin. Infect. Dis.* 56, 1445–1450.
- Beardmore, R.E., Pena-Miller, R., 2010. Rotating antibiotics selects optimally against antibiotic resistance, in theory. *Math. Biosci. Eng.* 7, 527–552.
- Bhagunde, P., Chang, K.T., Hirsch, E.B., Ledesma, K.R., Nikolaou, M., et al., 2012. Novel modeling framework to guide design of optimal dosing strategies for β -lactamase inhibitors. *Antimicrob. Agents Chemother.* 56, 2237–2240.
- Bjarnsholt, T., Jensen, P.O., Fiandaca, M.J., Pedersen, J., Hansen, C.R., et al., 2009. *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. *Pediatr. Pulmonol.* 44, 547–558.
- Bliss, C.I., 1939. The toxicity of poisons applied jointly. *Ann. Appl. Biol.* 26, 585–615.
- Boucher, H.W., Talbot, G.H., Benjamin, D.K., Bradley, J., Gidos, R.J., et al., 2013. 10 × '20 Progress—development of new drugs active against Gram-negative bacilli: an update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 56, 1685–1694.
- Box, G.E.P., Draper, N.R., 1987. *Empirical Model-Building and Response Surfaces*. Wiley, New York, pp. 424.
- Brown, E.M., Nathwani, D., 2005. Antibiotic cycling or rotation: a systematic review of the evidence of efficacy. *J. Antimicrob. Chemother.* 55, 6–9.
- Brown, S.P., Cornell, S.J., Sheppard, M., Grant, A.J., Maskell, D.J., et al., 2006. Intracellular demography and the dynamics of *Salmonella enterica* infections. *PLoS Biol.* 4, e349.
- Chait, R., Craney, A., Kishony, R., 2007. Antibiotic interactions that select against resistance. *Nature* 446, 668–671.
- Chou, T.C., 2006. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol. Rev.* 58, 621–681.
- Coates, A.R.M., Hu, Y., 2007. Novel approaches to developing new antibiotics for bacterial infections. *Br. J. Pharmacol.* 152, 1147–1154.
- Colijn, C., Cohen, T., Fraser, C., Hanage, W., Goldstein, E., et al., 2010. What is the mechanism for persistent coexistence of drug-susceptible and drug-resistant strains of *Streptococcus pneumoniae*? *J. R. Soc. Interface* 7, 905–919.
- Czock, D., Keller, F., 2007. Mechanism-based pharmacokinetic–pharmacodynamic modeling of antimicrobial drug effects. *J. Pharmacokinet. Pharmacodyn.* 34, 727–751.
- D'Agata, E.M.C., Dupont-Rouzeyrol, M., Magal, P., Olivier, D., Ruan, S., 2008. The impact of different antibiotic regimens on the emergence of antimicrobial-resistant bacteria. *PLoS ONE* 3, e4036.
- Davies, J., 2006. Where have all the antibiotics gone? *Can. J. Infect. Dis.* 17, 287–290.
- Dawson, C.C., Intapa, C., Jabra-Rizk, M.A., 2011. “Persisters”: survival at the cellular level. *PLoS Pathog.* 7, e100121.
- Defoirdt, T., Boon, N., Bossier, P., 2010. Can bacteria evolve resistance to quorum sensing disruption? *PLoS Pathog.* 6, e1000989.
- Deresinski, S., 2007. Principles of antibiotic therapy in severe infections: optimizing the therapeutic approach by use of laboratory and clinical data. *Clin. Infect. Dis.* 45 (Suppl. 3), S177–S183.
- Dethlefsen, L., Huse, S., Sogin, M.L., Relman, D.A., 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol.* 6, e280.
- Ehrlich, P., 1913. Address in pathology on chemotherapeutics. *Lancet* 182, 445–451.
- In: Pena-Miller, R., Laehnemann, D., Jansen, G., Fuentes-Hernandez, A., Rosenstiel, P., 2013. When the most potent combination of antibiotics selects for the greatest bacterial load: the smile-frown transition. *PLoS Biol.* 11, e1001540.
- Eswarappa, S.M., 2010. Location of pathogenic bacteria during persistent infections: insights from an analysis using game theory. *PLoS ONE* 4, e5383.
- Goutelle, S., Maurin, M., Rougier, F., Barbaut, X., Bourguignon, L., et al., 2008. The Hill equation: a review of its capabilities in pharmacological modelling. *Fundam. Clin. Pharmacol.* 22, 633–648.
- Greco, W.R., Bravo, G., Parsons, J.C., 1995. The search for synergy: a critical review from a response surface perspective. *Pharmacol. Rev.* 47, 331–385.
- Grobe, K.J., Zahler, J., Stewart, P.S., 2002. Role of dose concentration in biocide efficacy against *Pseudomonas aeruginosa* biofilms. *J. Ind. Microbiol. Biotechnol.* 29, 10–15.
- Hermesen, R., Deris, J.B., Hwa, T., 2012. On the rapidity of antibiotic resistance evolution facilitated by a concentration gradient. *Proc. Natl. Acad. Sci. U. S. A.* 109, 10775–10780.
- Højby, N., Bjarnsholt, T., Givskov, M., Molin, S., Ciofu, O., 2010. Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Agents* 35, 322–332.
- Houck, P.M., Bratzler, D.W., Nsa, W., Ma, A., Bartlett, J.G., 2004. Timing of antibiotic administration and outcomes for Medicare patients hospitalized with community-acquired pneumonia. *Arch. Intern. Med.* 164, 637–644.
- Iranzo, J., Perales, C., Domingo, E., Manrubia, S.C., 2011. Tempo and mode of inhibitor–mutagen antiviral therapies: a multidisciplinary approach. *Proc. Natl. Acad. Sci. U. S. A.* 108, 16008–16013.
- Jawetz, E., Gunnison, J.B., 1953. Antibiotic synergism and antagonism; an assessment of the problem. *Pharmacol. Rev.* 5, 175–192. In: Cokol, M., Chua, H.N., Tasan, M., Mutlu, B., Weinstein, Z.B. et al., 2011. Systematic exploration of synergistic drug pairs. *Mol. Syst. Biol.* 7:544.
- Johnson, M.D., MacDougall, C., Ostrosky-Zeichner, L., Perfect, J.R., Rex, J.H., 2004. Combination antifungal therapy. *Antimicrob. Agents Chemother.* 48, 693–715.
- Jonker, D.M., Visser, S.A., van der Graaf, P.H., Voskuyl, R.A., Danhof, M., 2005. Towards a mechanism-based analysis of pharmacodynamic drug–drug interactions in vivo. *Pharmacol. Ther.* 106, 1–18.
- Jumbe, N., Louie, A., Leary, R., Liu, W.G., Deziel, M.R., et al., 2003. Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy. *J. Clin. Invest.* 112, 275–285.
- Kardas, P., 2002. Patient compliance with antibiotic treatment for respiratory tract infections. *J. Antimicrob. Chemother.* 49, 897–903.

- Keren, I., Kaldalu, N., Spoering, A., Wang, Y., Lewis, K., 2004. *Persister cells and tolerance to antimicrobials*. FEMS Microbiol. Lett. 230, 13–18.
- Kirby, T., 2012. Europe to boost development of new antimicrobial drugs. Lancet 379, 2229–2230.
- Lambert, R.J., Johnston, M.D., Hanlon, G.W., Denyer, S.P., 2003. Theory of antimicrobial combinations: biocide mixtures – synergy or addition? J. Appl. Microbiol. 94, 747–759.
- Landersdorfer, C.B., Ly, N.S., Xu, H., Tsuji, B.T., Bulitta, B.J., 2013. Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design. Antimicrob. Agents Chemother. 57, 2343–2351.
- Lee, J.J., Kong, M., Ayers, G.D., Lotan, R., 2007. Interaction index and different methods for determining drug interaction in combination therapy. J. Biopharm. Stat. 17, 461–480.
- Levin, B.R., Udekwi, K.I., 2010. Population dynamics of antibiotic treatment: a mathematical model and hypotheses for time-kill and continuous-culture experiments. Antimicrob. Agents Chemother. 54, 3414–3426.
- Lewis, K., 2010. *Persister cells*. Annu. Rev. Microbiol. 64, 357–372.
- Lipsitch, M., Levin, B.R., 1997. The population dynamics of antimicrobial chemotherapy. Antimicrob. Agents Chemother. 41, 363–373.
- Loewe, S., 1953. The problem of synergism and antagonism of combined drugs. Arzneimittelforschung 3, 285–290.
- Lynch, S.V., Dixon, L., Benoit, M.R., Brodie, E.L., Keyhan, M., et al., 2007. Role of the rapA gene in controlling antibiotic resistance of *Escherichia coli* biofilms. Antimicrob. Agents Chemother. 51, 3650–3658.
- Macleane, R.C., Hall, A.R., Perron, G.G., Buckling, A., 2010. The evolution of antibiotic resistance: insight into the roles of molecular mechanisms of resistance and treatment context. Discov. Med. 10, 112–118.
- Martinez, L.R., Casadevall, A., 2006. *Cryptococcus neoformans* cells in biofilms are less susceptible than planktonic cells to antimicrobial molecules produced by the innate immune system. Infect. Immun. 74, 6118–6123.
- Mayer, H., Zaenker, K.S., van der Heiden, U., 1995. A basic mathematical model of the immune response. Chaos 5, 155–161.
- McKinnon, P.S., Davis, S.L., 2004. Pharmacokinetic and pharmacodynamic issues in the treatment of bacterial infectious diseases. Eur. J. Clin. Microbiol. 23, 271–288.
- Michalowicz, J., Nichols, J., Bucholtz, J., Olson, C., 2011. A general Isserlis theorem for mixed-Gaussian random variables. J. Stat. Phys. 136, 89–102.
- Michel, J.B., Yeh, P.J., Chait, R., Moellering, R.C., Kishony, R., 2008. Drug interactions modulate the potential for evolution of resistance. Proc. Natl. Acad. Sci. U. S. A. 105, 14918–14923.
- Nielsen, E.I., Cars, O., Friberg, L.E., 2011. Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. Antimicrob. Agents Chemother. 55, 4619–4630.
- Pena-Miller, R., Laehnemann, D., Jansen, G., Fuentes-Hernandez, A., Rosenstiel, P., et al., 2013. When the most potent combination of antibiotics selects for the greatest bacterial load: the smile-frown transition. PLoS Biol. 11, e1001540.
- Pena-Miller, R., Lahnemann, D., Schulenburg, H., Ackermann, M., Beardmore, R., 2012. The optimal deployment of synergistic antibiotics: a control theoretic approach. J. R. Soc. Interface 9, 2488–2502.
- Spicknall, I.H., Foxman, B., Marrs, C.F., Eisenberg, J.N.S., 2013. A modeling framework for the evolution and spread of antibiotic resistance: literature review and model categorization. Am. J. Epidemiol. 178, 508–520.
- Stewart, P.S., 2003. Diffusion in biofilms. J. Bacteriol. 185, 1485–1491.
- Torella, J.P., Chait, R., Kishony, R., 2010. Optimal drug synergy in antimicrobial treatments. PLoS Comput. Biol. 6, e1000796.
- Tosh, P.K., McDonald, L.C., 2012. Infection control in the multidrug-resistant era: tending the human microbiome. Clin. Infect. Dis. 54, 707–713.
- Udekwi, K., Levin, B.R., 2012. *Staphylococcus aureus* in continuous culture: a tool for the rational design of antibiotic treatment. PLoS ONE 7, e3866.
- van de Waterbeemd, H., Gifford, E., 2003. ADMET in silico modelling: towards prediction paradise? Nat. Rev. Drug Discov. 2, 192–204.
- Wang, J.Z., Nightingale, C.H., Sweeney, K.S., Xi, N.Z., Wang, D.M., 2003. An approach for the evaluation of synergy between antimicrobials. Int. J. Antimicrob. Agents 21, 274–278.
- Wood, K., Nishida, S., Sontag, E.D., Cluzel, P., 2012. Mechanism-independent method for predicting response to multidrug combinations in bacteria. Proc. Natl. Acad. Sci. U. S. A. 109, 12254–12259.
- Yeh, P.J., Hegreness, M.J., Aiden, A.P., Kishony, R., 2009. Drug interactions and the evolution of antibiotic resistance. Nat. Rev. Microbiol. 7, 460–466.
- Zhang, L., Mah, T.F., 2008. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J. Bacteriol. 190, 4447–4452.