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1 The ingredients for an antimicrobial mathematical modelling broth

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8 Abstract

9 Mathematical modelling has made significant contributions to the optimisation of the use of
10 antimicrobial treatments. In this review we discuss the key processes that such mathematical
11 modelling should attempt to capture. In particular, we highlight that the response of the host
12 immune system requires quantification and illustrate this with a novel model structure.

13 Keywords

14 Mathematical modelling, pharmacokinetics, pharmacodynamics, drug resistance.

15

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19 Introduction – a brief history of PKPD modelling

20 There is a long history of the investigation of optimal dosing regimens [1] that form the precursor to
21 the application of mathematical modelling (or PKPD) to the discovery and development of antibiotic
22 therapies. An understanding of optimal dose and frequency of dosing is now informed by the
23 understanding of pharmacokinetic and pharmacodynamic differences between drugs, between host
24 species and species and strains of pathogens. However, like many therapy areas, antibiotic PKPD
25 (exposure- response) modelling is often very empirical, the aim being to identify a target drug
26 concentration to test in patients. This is driven in part by necessity and gaps in our understanding. A
27 concern with this approach might be that important mechanisms are not addressed which may limit
28 quantitative translation to the clinic - especially the translation of optimum dose and dosing
29 schedule. This is because the empirical approach may not fully account for time dependent factors
30 such drug concentration (PK), bacterial load (disease course) drug resistance and the response of the
31 immune system. Below we exemplify that a mathematical model, developed to capture key
32 antibiotic PKPD mechanisms, need not be complex and yet can provide further insight into the
33 biology behind the experimental data. Many of these aspects are thoroughly reviewed already in
34 Nielsen and Friberg 2013 [2] and Rayner et al 2021 [3] so will not be covered in detail here – the aim
35 in this paper will be to identify the key components or “ingredients” required in a mechanistic model
36 of antimicrobial drug action, in particular the response of the immune system.

37 First Model Ingredient: Exposure response

38 The first requirement is an understanding of the exposure-response relationship and how it
39 translates from in vitro to in vivo mouse models. This is important for dose setting in the clinic with
40 the addition of pharmacokinetic knowledge. Demonstrating translation of the exposure-response
41 relationship from in vitro to in vivo allows a much wider range of species of bacteria to be considered
42 than an in vivo resource would afford. Historically the minimum inhibitory concentration (MIC) has
43 been used as the in vitro potency measure. To determine the aspect of drug exposure most
44 predictive of antimicrobial activity the in vivo free AUC/MIC, C_{max}/MIC and time over MIC are
45 plotted versus the reduction in bacterial load in vivo for a range of antibiotic dose levels. Potentially
46 this is carried out in multiple strains of bacteria with MIC being used to normalise drug exposure for
47 inherent susceptibility. Dose-fractionation is a necessary study design element because of the
48 inherent correlation of C_{max}, AUC and time over MIC as dose is varied. A more serious consideration
49 is that MIC is a composite potency measure influenced not only by the pharmacological effect of the
50 drug (reduced proliferation/ killing) but also the intrinsic proliferation rate of the bacteria and
51 background death rates [2]. It is also potentially dependent on the duration of the drug incubation
52 and cell density used in the assessment. Typically, these metrics suggest that maintaining exposure
53 above MIC is required for a reduction in infection – and this seems rational given that in vitro
54 concentrations above MIC, by definition, will reduce the population of bacteria. An example of this is
55 colistin [4], where free AUC/MIC >10 (Average concentration 10-fold that of MIC) are required to
56 reduce the CFU count.

57 There are more mechanistic approaches that have been adopted to characterise the course of
58 infection and concentration-effect relationship as thoroughly reviewed by [Nielsen and Friberg
59 2013]. These models, applied to time series data from in vitro and in vivo experiments, separate the
60 intrinsic growth rate and an EC₅₀, that relates drug concentration to effect, that that will not suffer
61 from the potential oversimplification of MIC. This is very important given that in vivo
62 pharmacokinetics result in significant fluctuations in the drug concentration that the infection is
63 exposed to, compared to the constant concentrations typical of an in vitro incubation. However, it is
64 noted that systems such as hollow fibre injection and other dynamic in vitro models can reproduce

65 fluctuating drug concentrations and provide a useful link between in vitro and in vivo experiments.
66 Similarly time-kill experiments give insight into the onset of antibacterial effect.

67 Combination therapy is one way that antimicrobial resistance might be circumvented. Typically two
68 or more antimicrobials are investigated in a concentration dependent manner, similar to the
69 determination of MIC, and the data tested for evidence of greater or less than additive effect using
70 the concepts of Bliss independence and Loewe additivity[5]. An more general approach to modelling
71 combination effects has been proposed [6]. Doern [7] has argued that in vitro combination assays
72 are so diverse that assessing synergy and guiding dosing of patients with these assays is a non-
73 starter until a gold standard is agreed upon. As argued against MIC, these static approaches may not
74 pull apart the contributing factors that contribute to combination pharmacology and the
75 dependence on the test system such as the population growth rate. More mechanistic approaches
76 have been taken, usually borrowing assumptions of combination effects familiar in other therapy
77 areas such as an additive effect of the total bacterial kill. A review of these models [8] concluded
78 that there was a benefit to mechanistic approaches in being able to not only disentangle the
79 contribution of components but also to incorporate host associated effects, e.g., the immune
80 response.

81 [Second Model Ingredient: The development of drug resistance](#)

82 A second requirement is an understanding of the kinetics of drug resistance – especially important
83 given the growing issues of AMR. Key resistance mechanisms include: (i) changes to the structure of
84 the drug target; and (ii) increased expression of proteins that alter the intracellular PK of the
85 antibiotic (drug transporters and drug metabolising enzymes). The former tends to be irreversible,
86 requiring an alteration at the gene level, however the latter can be reversible if an environmental
87 adaption occurs. MIC, taken after a particular time, may well have these aspects folded in. However,
88 resistance, and its impact on the time course of an infection will be time dependent. Models based
89 on time series data can incorporate these mechanisms and to some extent again separate them out
90 from inherent potency and population growth rate. Key phenotypes to incorporate are [9] :
91 resistance from the start, tolerance - whereby cells adapt to a reversibly resistant phenotype, and
92 persisters - which have a lower rate of proliferation and so are less vulnerable to typical mechanisms
93 of antimicrobial treatments[10]. These can all be incorporated [2] and permit the prediction of
94 unique time-kill curves so that the underlying phenotypes might be inferred. Distinguishing between
95 mechanisms from a numerical point of view suggests that this might be possible based upon
96 bacterial counts only however challenging if attempting to distinguish between resistance
97 phenotypes simultaneously [11]. Experimental approaches to aid in this identification have been
98 suggested including measuring the MIC and MDK (minimum duration for killing) in the resulting
99 resistant populations[9].

100 An issue for ongoing research in this area is the lack of diverse data sets considered. Niewiadomska
101 et al [12] reviewed the literature and found a lack of diversity in pathogenic organisms in which AMR
102 had been mathematically modelled and calibrated on experimental data, so clearly further work is
103 required to fully validate the above mathematical mechanisms. It is also noted that these
104 mathematical modelling exercises have not considered the combination of multiple treatments.

105 [Third Model Ingredient: The contribution of the immune system to cure](#)

106 The impact of the immune system has previously been considered in other therapy areas, for
107 example in oncology. Here models have attempted to capture the immune system's recognition of
108 malignant cells, the onset of response and, in some cases, the attempts of the tumour to escape or
109 adapt to this immune response [13-15]. It is evident that the immune response's contribution to the

110 clearance of an infectious agent is important. Studies in animal infection models [16, 17] have, to
 111 some extent, quantified the immune component of clearance. Clearly then, there is an analogy with
 112 oncology, suggesting the extension of antibiotic PKPD to this approach could be of use in the
 113 development and optimisation of therapies.

114 Developing a model of within-host antibiotic resistance which accounts for the role of the immune
 115 system is cursed by the overall complexity of the biological system and the inherent multiscale
 116 nature of the systems (molecular to whole-body scale). However, it is possible to create a generic
 117 description of each aspect of the system – bacterial loading and the immune system response to it
 118 whilst accounting for the administration of an antibiotic. We describe here a within-host population
 119 mathematical model which accounts for bacterial loading and clearance via the immune system and
 120 an antibiotic. Here we take a generalised view of the immune system accounting for the speed and
 121 magnitude of the immune response, which we assume responds to the bacterial infection, both in
 122 terms of increasing the response and its magnitude. Our model formulation accounts for the local
 123 and global effects of antibiotic dosing as summarised in Figure 1. Mathematically our model is
 124 represented by the three nonlinear ordinary differential equations given by

$$\begin{aligned}
 125 \quad \frac{dA}{dt} &= \overbrace{\alpha(t)}^{\text{Antibiotic dosing}} - \overbrace{\lambda_A AB}^{\text{Antibiotic removal by bacteria}} - \overbrace{\delta_A A}^{\text{Antibiotic elimination}}, \\
 126 \quad \frac{dB}{dt} &= \overbrace{\rho_B B \left(1 - \frac{B}{K_B}\right)}^{\text{Bacterial growth}} - \overbrace{\frac{\lambda_B AB}{K_R + A}}^{\text{Antibiotic removal}} - \overbrace{rIB}^{\text{Immune system removal}}, \\
 127 \quad \frac{dI}{dt} &= \overbrace{\rho_I (1 + s_g B) \left(1 - \frac{I}{K_I(1 + s_I B)}\right)}^{\text{Immune system response}} - \overbrace{\delta_I I}^{\text{Immune clearance}}
 \end{aligned}$$

128 where the initial conditions of the system are given by

$$129 \quad A(0) = 0, \quad B(0) = B_0 \quad \text{and} \quad I(0) = I_0.$$

130 Here $A = A(t)$ denotes the concentration of the antibiotic, $B = B(t)$ the within host bacterial cell
 131 density and $I = I(t)$ the magnitude of the immune response. It is assumed that both the bacteria
 132 and immune system response grow logistically, the latter with growth rate $\rho_I(B) = \rho_I(1 + s_g B)$ and
 133 carrying-capacity $K_I(B) = K_I(1 + s_I B)$, where s_g and s_I describe how the speed and magnitude of
 134 the immune system response respond to the bacterial infection. Here the effect of antibiotic
 135 resistance is accounted for by modelling the antibiotic concentration effect on bacteria via a
 136 sigmoidal function with half-maximal value K_R (a large K_R means the antibiotic has reduced potency
 137 and so the bacteria will be relatively resistant).

138 To simplify the three-dimensional nature of equations (1) to (3) we consider the global effect of the
 139 antibiotic by ignoring the localised dependency described via the term $\lambda_A AB$, so henceforth, for this
 140 work, we set $\lambda_A = 0$. This decouples equation (1) from equations (2) and (3) thus allowing us to
 141 analyse equations (2) and (3) as a system of two coupled nonlinear ODEs, which we do so using the
 142 non-dimensionalised form of the equations as detailed in Annexe 1. Under the assumption of a
 143 constant antibiotic infusion ($\alpha(t) = \alpha$), the system exhibits four steady-states:

144 (i) **State 1** $(a_1^*, b_1^*, i_1^*) = (\alpha^*, 0, 0)$: the case in which the host has died, all bacteria have been
 145 eradicated from the body and only antibiotic remains;

146 (ii) **State 2** $(a_2^*, b_2^*, i_2^*) = \left(a^*, 0, \left(1 - \frac{\delta_i}{\rho_i} \right) \right)$: All bacteria have been eradicated from the body,
147 the immune system has returned to its normal functional levels and antibiotic remains in the
148 system so long as the immune system response is greater than its clearance;

149 (iv) **State 3**: $(a_3^*, b_3^*, i_3^*) = \left(a^*, \left(1 - \frac{(\lambda_b^* + i^*)}{\rho_b} \right), i^* \right)$: A co-existence steady-state in which both
150 the antibiotic and immune system work together to eradicate the bacterial loading, but the
151 infection persists (i^* being given by the solution of equation (A.2)); and

152 (iii) **State 4** $(a_4^*, b_4^*, i_4^*) = \left(a^*, \left(1 - \frac{\lambda_b^*}{\rho_b} \right), 0 \right)$: Here a persistent bacterial infection remains in
153 the body along with the antibiotic, the immune system effectively having become non-
154 functional,

155 where $a^* = \alpha / \delta_a$ is the steady-state antibiotic concentration and $\lambda_b^* = \lambda_b a^* / (K_r + a^*)$. State 1 is
156 possible (stable) if the immune system clearance is more rapid than its response rate ($\delta_i > \rho_i$),
157 whilst the reverse holds for State 2 with the additional condition that $\rho_b + \frac{\delta_i}{\rho_i} < 1$. State 3 is
158 monotonically or damped oscillatory stable and State 4 is stable so long as bacterial growth
159 dominates over the ability of the antibiotic to remove the bacteria ($\rho_b > \lambda_b^*$).

160 States 1 and 2 represent the worst and best health outcomes, whilst State 3 is a common scenario
161 which is representative of antibiotic resistance. State 4 is the case of a severely immune-suppressed
162 individual. In what follows we focus primarily on scenarios considering Cases 2 and 3 given these
163 represent more likely health outcomes.

164 To demonstrate the dynamical behaviour of the system we consider numerical solutions, generated
165 in Matlab, of equations (1) to (3) utilising the set of non-dimensional parameters stated in Table 1.
166 We have chosen parameterisations here which allow us to reflect on different scenarios informed by
167 real-world known outcomes. Our objective here is to demonstrate the conceptual qualitative nature
168 of the system, and how a simplified description of the respective biological mechanisms can be used
169 to capture the gross behaviour of the system, without needing to describe all aspects of the
170 underlying biology. Such models allow for the overall system dynamics to be explored before
171 understanding aspects of the lower-level detail. A non-dimensionalisation allows us to inform
172 parameter relationships in order to reproduce known qualitative behaviour. Experimental and
173 clinical parameterisation of the system will be the focus of future work.

174 Case studies of simulations are shown in Figure 2. We first consider the ability of the immune system
175 to clear the bacterial infection in the absence of antibiotic. This allows us to parameterise the system
176 for an individual whose immune system is strong enough to clear the infection, as detailed in Figure
177 2a, for the parameterisation given in Table 1. This is akin to State 2 above, albeit that no antibiotic is
178 present.

179 We next consider the case of an immune system which is slower in responding to the presence of
180 bacteria ($\rho_i = 0.07$). Here the individual is not able to effectively clear the infection and, after an
181 initial period of oscillations between the immune system and bacterial loading, the system settles to
182 a non-zero steady-state. This is akin to the co-existence steady-state (State 3), albeit in the absence
183 of any antibiotic. We now consider how we can utilise an antibiotic to support the removal of the
184 bacteria from the system and thus move it from State 3 to State 2. We do so by first introducing an
185 antibiotic with a perceived level of effectiveness (as indicated by λ_b) in Figure 2(c) with $\alpha = 1$, $\rho_i =$
186 0.07 and $\lambda_b = 0.1$. Here we see that the antibiotic is able to decrease the bacterial loading, but is

187 not fully effective. Increasing the effectiveness of the anti-biotic (akin to adding additional
188 antibiotics; $\lambda_b = 0.25$) leads to effective removal of the bacteria thus moving the system to the ideal
189 outcome of State 2.

190

191 Conclusions

192 In this article, we have reviewed the three main ingredients required of a mechanistic PKPD model:
193 exposure-response, drug sensitivity/ resistance phenotypes and the contribution of the immune
194 system. In particular, we have highlighted how quantifying the immune system response can aid in
195 the interpretation of in vitro to in vivo translation of disease pharmacology. Indeed, we should
196 perhaps consider it as modelling the drug's contribution on top of the immune system: By increasing
197 the clearance of pathogen the immune system is able to fully respond. Further work is needed in this
198 area including informative measurement of the host immune system.

199 By having these three aspects it is possible that the mathematical models can account for and
200 explain between-host variations in the time course of infection as well as the potential to be more
201 translatable from nonclinical systems to patients. The relationship between regimen and efficacy can
202 vary in terms of the pharmacokinetics, bacterial strain (MIC), adaption/resistance (variation of MIC
203 with time) and the immune response. By factoring these in, the intrinsic factors determining the
204 success of treatment can be identified by building patient baseline covariates into the model. This
205 can aid in the optimisation of antimicrobial treatment. Key to this is the application of mechanistic
206 models that can be applied in a nonlinear mixed effects framework to characterise between-subject
207 variability in response – and it is possible the above model is applicable here. Most promising is the
208 ability of the model to quantify the immune system response and perhaps here there is some
209 overlap in the work quantifying the efficacy of vaccines. Clearly, however, full mathematical
210 evaluation and experimental data that allow determination of model parameters to support model
211 validation are needed for future work.

212

213 **Annexe 1 – Non-dimensional governing equations**

214 Equations (1) to (3) are non-dimensionalised according to

215
$$A(t) = K_B a(\tau), \quad B(t) = K_B b(\tau), \quad I(t) = K_I i(\tau) \quad \text{and} \quad t = \frac{\tau}{r K_I}.$$

216 Substituting these scalings leads to the non-dimensional system of equations

217
$$\frac{da}{d\tau} = \overbrace{\alpha(\tau)}^{\text{Antibiotic dosing}} - \overbrace{\lambda_a ab}^{\text{Antibiotic removal by bacteria}} - \overbrace{\delta_a a}^{\text{Antibiotic elimination}},$$

218
$$\frac{db}{d\tau} = \overbrace{\rho_b b(1-b)}^{\text{Bacterial growth}} - \overbrace{\frac{\lambda_b ab}{K_r + a}}^{\text{Antibiotic removal}} - \overbrace{\widehat{ib}}^{\text{Immune system removal}},$$

219
$$\frac{di}{d\tau} = \overbrace{\rho_i(1 + \varepsilon_g b) \left(1 - \frac{i}{1 + \varepsilon_i b}\right)}^{\text{Immune system response}} - \overbrace{\widehat{\delta_i i}}^{\text{Immune clearance}},$$

220 where the initial conditions of the system are given by

221
$$a(0) = a_0, \quad b(0) = b_0 \quad \text{and} \quad i(0) = i_0,$$

222 and the non-dimensional parameters by

223
$$\alpha(\tau) = \frac{\overline{\alpha(t)}}{K_B K_I r}, \quad \lambda_a = \frac{\lambda_A K_B}{r K_I}, \quad \delta_a = \frac{\delta_A}{r K_I}, \quad \rho_b = \frac{\rho_B}{r K_I}, \quad \lambda_b = \frac{\lambda_B}{r K_I}, \quad K_r = \frac{K_R}{K_B}, \quad \rho_i = \frac{\rho_I}{r K_I},$$

224
$$\delta_i = \frac{\delta_I}{r K_I}, \quad \varepsilon_g = s_g K_B \quad \text{and} \quad \varepsilon_i = s_I K_B.$$

225 **Annexe 2 – Co-existence steady-state solution**

226 The third co-existence steady state (a_3^*, b_3^*, i_3^*) is determined by solving

227
$$\frac{\varepsilon_g}{\rho_b} \left(1 + \frac{\varepsilon_i}{\rho_b}\right) i^{*2} - \left[\frac{\varepsilon_i}{\rho_b} \left(2\varepsilon_g - \frac{\delta_i}{\rho_i}\right) + \varepsilon_g \left(1 + \frac{1}{\rho_b}\right) + 1 + \frac{\varepsilon_i}{\rho_b} \right] i^* + (1 + \varepsilon_i) \left(\varepsilon_g + 1 - \frac{\delta_i}{\rho_i}\right) = 0, \quad \dots \text{(A2)}$$

228 for i^* . We observe that positive solutions are only possible here for $i^* < \rho_b - \lambda_b^*$, i.e. the immune
 229 system levels are determined by the difference in the bacterial growth rate and its rate of
 230 eradication by the antibiotic.

231 **Figure Legends**

232 **Figure 1. A schematic of the three-state model of within host antimicrobial resistance.** Here an
 233 antibiotic $A(t)$ is administered at rate $\alpha(t)$ and cleared with rate constant δ_A . Bacteria $B(t)$ grow
 234 logistically with growth rate constant ρ_B and are removed by the antibiotic (with rate constant λ_A)
 235 and the immune system $I(t)$ (with rate constant λ_B), respectively. Bacteria seek to inhibit the
 236 immune system, which seeks to respond with growth rate $\rho_I(B)$ by increasing its capacity $K_I(B)$, to
 237 clear bacteria with rate constant r , whilst being removed with rate constant δ_I .

238 **Figure 2. Case studies of the antimicrobial resistance model.** Solid lines indicate the within host
 239 bacterial cell density, whilst dotted lines the immune system response. Antibiotic concentration not
 240 shown. **(a)** The case of a strong immune system, in the absence of any bacteria, being able to clear a
 241 bacterial infection ($\alpha = 0, \rho_i = 0.1$). **(b)** An immune system which responds less rapidly than (a),
 242 which leads to oscillatory damped behaviour and the bacteria not being effectively removed from the
 243 host ($\alpha = 0, \rho_i = 0.07$). **(c)** The effect of including an antibiotic for (b) to help remove the bacterial

244 infection. Here the infection still persists after antibiotic has been included ($\alpha = 1, \rho_i = 0.07, \lambda_b =$
245 0.1). **(d)** In contrast to (c) a more effective antibiotic is able to remove the bacterial infection, leaving
246 the immune system to return to its pre-infection levels ($\alpha = 1, \rho_i = 0.07, \lambda_b = 0.25$).

247

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