A computational model of antibiotic-resistance mechanisms in Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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**Abstract**

An agent-based model of bacteria–antibiotic interactions has been developed that incorporates the antibiotic-resistance mechanisms of Methicillin-Resistant Staphylococcus aureus (MRSA). The model, called the Micro-Gen Bacterial Simulator, uses information about the cell biology of bacteria to produce global information about population growth in different environmental conditions. It facilitates a detailed systems-level investigation of the dynamics involved in bacteria–antibiotic interactions and a means to relate this information to traditional high-level properties such as the Minimum Inhibitory Concentration (MIC) of an antibiotic. The two main resistance strategies against β-lactam antibiotics employed by MRSA were incorporated into the model: β-lactamase enzymes, which hydrolytically cleave antibiotic molecules, and penicillin-binding proteins (PBPs2a) with reduced binding affinities for antibiotics. Initial tests with three common antibiotics (penicillin, ampicillin and cephalothin) indicate that the model can be used to generate quantitatively accurate predictions of MICs for antibiotics against different strains of MRSA from basic cellular and biochemical information. Furthermore, by varying key parameters in the model, the relative impact of different kinetic parameters associated with the two resistance mechanisms to β-lactam antibiotics on cell survival in the presence of antibiotics was investigated.

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1. Introduction

The emergence of multi-drug resistance in *Staphylococcus aureus* bacteria has become a major healthcare problem in recent years. Before the introduction of antibiotics, patients with *S. aureus* bacteraemia had a mortality rate of over 80% (Skinner and Keefer, 1941). This situation improved dramatically when the first β-lactam antibiotic, penicillin, was introduced into clinical use during the early 1940s (Chalm et al., 1993). However, today greater than 95% of all *S. aureus* isolates possess resistance to penicillin, and 40–60% of clinical isolates in the United States of America and the United Kingdom express methicillin resistance (Levy and Marshall, 2004; Neu, 1992). The introduction of methicillin in 1959 to treat infections of penicillin-resistant *S. aureus* resulted in the selection of Methicillin-Resistant *S. aureus* (MRSA) strains (Fudal et al., 2005). As early as 1961, MRSA strains were isolated and there has been a steady increase in incidences since then (Eiksen, 1961).

The mediator of penicillin resistance in *S. aureus* is a β-lactamase enzyme, which hydrolytically cleaves the β-lactam ring present in this antibiotic. β-Lactamase was first discovered in *Escherichia coli* bacteria in 1940, and β-lactamase-expressing *S. aureus* bacteria were isolated soon afterwards (Abraham and Chain, 1940; Bondi and Dietz, 1945; Kirby, 1944). The appearance of β-lactamases is thought to be an ancient evolutionary event. However, their broadened distribution across many bacterial species, under selective pressure from antibiotic usage, has become a serious health concern (Fisher et al., 2005).

Four variants (Types A–D) of *S. aureus* β-lactamase have been identified by immunologic methods (Zygmun et al., 1992). The most well-studied β-lactamase is the class A β-lactamase, characteristic of *S. aureus* strain PC1 (Ambler, 1975). This is encoded by the *blaZ* gene, which is carried on a transposable element of a large plasmid. Expression of β-lactamase is regulated by the interaction between β-lactams in the environment and a cell surface signal-transducer protein BlaR1 (Fudal et al., 2005; Lewis et al., 1999). After induction of expression, most of the β-lactamase enzyme is secreted into the extracellular milieu, while some remain bound to the cytoplasmic membrane of the
cell (Nielsen and Lampen, 1982). When the antibiotic concentration in the environment decreases, re-expression of β-lactamase expression occurs because BlaR1 is no longer auto-activated (Zhang et al., 2001). A study by Norris et al. (1994) found that among 50 β-lactamase-producing MRSA isolates taken from nine locations across the USA, 80% expressed Type A β-lactamase and the remainder expressed Type C, Type B and Type D β-lactamases are thought to be less common among MRSA strains (Norris et al., 1994).

Normal S. aureus cells produce four types of membrane-bound transpeptidase proteins called penicillin-binding proteins (PBPs 1–4), which assemble and regulate the final stages of cell wall biosynthesis. The mode of action of the β-lactam antibiotics involves binding to and inhibiting the transpeptidase (cell wall cross-linking) activity of the PBPs. However, MRSA bacteria contain a gene called meca, which encodes an extra penicillin-binding protein, PBP2a. PBP2a does not bind the β-lactam moiety readily because the approach to the active site is sterically encumbered. When an MRSA organism is subjected to β-lactam stress, PBP2a confers resistance by contributing its transpeptidase activity (cell wall cross-linking) to the transglycosylase function of native PBPs during cell wall synthesis (Fuda et al., 2005).

Mathematical population models are commonly used to describe the growth and development of a bacterial colony as a unit, using global parameters or state variables (Grimson and Barker, 1994; Lacasta et al., 1999). These “top-down” approaches have the advantage that they are computationally efficient and less parameter rich than more low-level approaches. An important limitation of the state variable approach is that it does not allow the user to trace back the system behaviour to the behaviour of the individual agents. For example, this approach cannot explain the underlying factors that lead to the population exhibiting a particular growth rate or carrying capacity (Grimm, 1999). However, they are important to provide an appropriate integrated view of the population behaviour.

Cellular automata theory has also been used to explain pattern formation in colonies (Ben-Jacob et al., 1994). However, an alternative approach to modelling bacterial growth and development is the agent-based (or individual-based) modelling approach (Ginovart et al., 2002; Kreft et al., 1998). The distinguishing characteristic of the agent-based approach is that the properties of the individual cells, rather than the colony as a whole, are used to determine the model. This “bottom-up” approach allows a finer grained analysis, connecting local changes at the cellular level to the overall patterns of population growth.

It must be noted that the agent-based approach and higher level mathematical approaches are not mutually exclusive but rather complement each other in studies of population dynamics. The latter approach allows a general conceptual framework to be developed for a population, which can lead to theories at the systems level. The agent-based approach meanwhile allows important features of the individuals to be taken into account and related to the overall system's properties.

The ‘Micro-Gen Bacterial Simulator’ was developed to model the growth and interactions of bacterial cells with antibiotics in vitro using the agent-based approach (Murphy and Walsh, 2007). The theory of autonomous agents is a useful approach for the modelling of bacterial cell colonies as it allows large-scale population models to be derived from simple rules dictating the growth and interactions of the individual components (bacterial cells) of the population. The Micro-Gen Bacterial Simulator uses information about the cell biology of bacteria to produce global information about population growth in different environmental conditions (Murphy and Walsh, 2007).

An agent-based approach was chosen over a simpler mass action model in order to explicitly model the heterogeneity in environmental conditions, for example between the interior and exterior of the colony and between individual bacterial cells. In complex microbial communities, such as biofilms, there can be highly heterogeneous localised niches where the chemistry varies dramatically over small distances. A principal future aim of the Micro-Gen project will be to expand the model to represent these more complex three-dimensional communities. Micro-Gen provides a robust framework to model these structures and explains how their complexity emerges from the interactions of simple autonomous microbial agents.

This paper documents the results of integrating antibiotic-resistance mechanisms into the bacterial agents, representing MRSA, and analyses the system dynamics of its interactions with some common β-lactam antibiotics. The aim of this study is to quantify the effects of the principal pharmacokinetic parameters of these antibiotics on treatment outcome, and assess their impact in terms of some common high-level measures of antibiotic efficacy such as the Minimum Inhibitory Concentration (MIC). It is important to explore the complex relationship between the pharmacokinetics of drugs and emergent pharmacodynamic parameters, such as the MIC, for the rational development of drug treatment regimes (Regoes et al., 2004). This provides a basis for understanding the dynamics involved in the development of antibiotic resistance, so as to be able to develop strategies to limit its expansion. Further work will be required to model the more complex dynamics found in the in vivo clinical setting, but as it stands, Micro-Gen represents a good theoretical framework for analysing the in vitro situation.

2. Model description

The Micro-Gen Bacterial Simulator is coded in the C# object-oriented programming language (Murphy and Walsh, 2007). It is an extended and modified version of a Java software tool, called BAT, previously developed in this laboratory (Walsh, 2006). Each bacterial cell of a colony is represented by a software agent with variables storing basic information such as energy state, and rules governing behaviour and interactions with other agents. The emergent properties of the population can be examined without the need for population-level (global) laws (Jennings et al., 1998). For example, by applying kinetic rules for the interactions of individual bacteria with antibiotic molecules, the concentration of antibiotic that leads to inhibition of growth of the entire colony can be derived as an emergent property. Attributes of the bacterial agents and the culture environment are configured by modifying a set of input parameters, which are summarised in Table 1.

For this study, the model was configured to represent MRSA bacteria growing in agar plate culture. Parameters applicable to three types of MRSA bacteria were used, which are differentiated by their β-lactamase status: Type A MRSA and Type C MRSA are named because of the type of β-lactamase enzyme they produce; and a β-lactamase-negative strain was also included. Type A and Type C β-lactamase enzymes are differentiated by their kinetic parameters (kcat/ Km) (see Table 1; Section 2.2).

The culture environment consists of a discrete, two-dimensional grid of “patches” which each contain specified levels of nutrients, enzymes and antibiotic molecules. The model allows for spatial heterogeneity in nutrient content and the distribution of bacteria, as opposed to assuming a completely homogeneous, mixed environment. Free molecules are subject to diffusion by a discretised implementation of Fick's First Law of diffusion (Ginovart et al., 2002). The concentration difference multiplied by a user-defined diffusion coefficient is used to determine the amount of substance that diffuses between two adjacent patches.
Table 1
Input parameters for Micro-Gen simulations of MRSA bacteria and antibiotics in agar plate culture

<table>
<thead>
<tr>
<th>Type of entity</th>
<th>Parameter (units)</th>
<th>Input value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>Patch area (b.u.)</td>
<td>20,000</td>
</tr>
<tr>
<td></td>
<td>Patch nutrient level (b.u.)</td>
<td>80,000</td>
</tr>
<tr>
<td></td>
<td>Diffusion coefficient</td>
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<tr>
<td>Bacterial agent</td>
<td>Bacterial generation time (min)</td>
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</tr>
<tr>
<td></td>
<td>Biomass threshold for division (b.u.)</td>
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</tr>
<tr>
<td></td>
<td>Nutrient intake (b.u./loop)</td>
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</tr>
<tr>
<td></td>
<td>Survival cost (b.u./loop)</td>
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</tr>
<tr>
<td></td>
<td>Stationary phase relative to metabolic rate</td>
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</tr>
<tr>
<td></td>
<td>Lag phase length (min)</td>
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</tr>
<tr>
<td></td>
<td>$\beta$-Lactamase production rate (pM/loop)</td>
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</tr>
<tr>
<td></td>
<td>Type A</td>
<td>$1.2 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>Type C</td>
<td>$1.2 \times 10^{-8}$</td>
</tr>
<tr>
<td></td>
<td>$\beta$-Lactamase production cost (b.u.)</td>
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</tr>
<tr>
<td>Antibiotic</td>
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<td>Penicillin G</td>
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<td></td>
</tr>
<tr>
<td>Ampicillin</td>
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<td></td>
</tr>
<tr>
<td>Cephalaxin</td>
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<td></td>
</tr>
<tr>
<td>Half-life (h)</td>
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<td></td>
</tr>
<tr>
<td>$k_{10}$ (s$^{-1}$)</td>
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<tr>
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<tr>
<td>Cephalaxin</td>
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<td>$K_{10}$ (pM)</td>
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<tr>
<td>Cephalaxin</td>
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<td>$K_{9}$ (s$^{-1}$)</td>
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<td>PHP2A</td>
<td>$K_{9}$ (pM)</td>
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<tr>
<td>Penicillin G</td>
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<tr>
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<tr>
<td>Cephalaxin</td>
<td>5.2</td>
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</tr>
</tbody>
</table>

*a* b.u., biomass units.

(b) loop (of simulation) = 1.98 s real time.

Distributed computing clusters. The environment of the culture is divided equally among the different computing nodes, with quantities such as nutrient, antibiotic and enzyme levels as well as bacterial agents communicating between nodes when they cross the overlapping boundary conditions. Tests have shown the algorithm to have a parallel efficiency of >90% when run in parallel on a 16 × 2.8 GHz Pentium 4 computing cluster (Murphy and Walsh, 2007).

2.1. Programme structure

The programme flow structure of Micro-Gen is displayed schematically in Fig. 2. At the beginning of the simulation, a bacterial “fabric” is created, which is an array of bacterial software agents in memory that acts as a pool from which new agents are derived. These agents are flagged as either “alive” or “dead”, to represent whether they are active in the simulation or not. The use of a fabric of pre-initialised agents, such as this, results in significant performance benefits as new software objects do not have to be created and destroyed in memory each time reproduction occurs. Instead, the programme searches for a “dead” agent in the bacterial fabric and updates its status to “alive” along with other relevant parameters.

During the main loop of the simulation, the various functions of the agents are carried out such as feeding, diffusion, etc. The temporal granularity of the simulation is determined by the time scale of the loop as each traversal represents one discrete time step. The programme loop was configured to represent ~2 s of real time, which corresponds to a simulated bacterial doubling time of 29 min for the MRSA agents under optimal conditions (Ender et al., 2004).

The real-time equivalent was calculated by running the simulation of the bacterial agents under optimal growth conditions and finding their average doubling time (number of loops) during the exponential phase of growth. This was then divided by the experimentally determined doubling time (29 min) of MRSA to find the real-time equivalent for each traversal of the programme loop. Other time-dependent model parameters, such
as the kinetic parameters (e.g., enzyme turnover rate $k_{\text{cat}}$, given later), were then calibrated to this specified time scale.

The first step of the programme loop is when the diffusion algorithm (Fick's First Law) is applied to the free molecules in the environment—nutrients, antibiotics and $\beta$-lactamase enzymes. Once the free molecule levels in each patch have been updated, the next step is to subtract the survival cost from the energy reserves of the bacterial agents. This represents energy expended by normal metabolic processes in the cell each time step.

Under conditions of nutrient deprivation, the bacterial cell's internal stock will decrease gradually over time as energy is burned off by standard metabolic activities. If it drops below a critical level, the cell may enter the 'stationary phase' which is a state in which the metabolic rate is decreased as the cell enters survival mode (Nystrom, 2001; Siegel and Kolter, 1992). If no nutrient is supplied, however, cell lysis will eventually occur when the nutrient stock reaches zero, and trace amounts of nutrient from the dead cell will be returned to the environment (0.1% of energy reserve of newly divided cell).

The movement stage (step 3) is when the cell-shunting algorithm is applied to bacterial agents in overcrowded patches. Step 4 of the programme loop is when interactions between bacterial cells, antibiotics and $\beta$-lactamase enzymes in the same patch are carried out according to defined kinetic parameters (given later). The bacterial agents can feed off available nutrient in the environment (step 5) and add to their internal energy reserve, or "stock". If their internal stock surpasses a defined threshold for reproduction then cell division, by binary fission, is initiated and two new daughter cell agents are produced. The programme continues to traverse this loop until there are no more alive agents or until a specified number of loops have elapsed.

2.2. MRSA-resistance mechanisms

$\beta$-Lactamase enzyme expression is induced when bacterial agents interact with antibiotic in the same patch, and there is a metabolic cost associated with $\beta$-lactamase production. A true/false flag for $\beta$-lactamase expression is associated with each
bacterial agent. After the first interaction (when the flag is changed to true), there is an exponential increase in the \( \beta \)-lactamase production rate until the maximum rate is reached after approximately 80 min (Lewis et al., 1999). This corresponds to the activation of gene expression mediated by antibiotic binding to the signal-transducer protein BlaR1, as described in Section 1. \( \beta \)-Lactamase is released into the local patch where it is subject to diffusion according to Fick's First Law, as described earlier. It also has a defined half-life parameter (see Table 1), determining its rate of degradation over time in the environment. When antibiotic is no longer present in the immediate environment of the bacterial agent then re-repression of \( \beta \)-lactamase expression occurs (BlaR1 is no longer auto-activated-software flag changed to false).

The interactions between \( \beta \)-lactamases and antibiotics in the environment are defined by Michaelis–Menten kinetics, whereby the reaction rate \( V \) is determined by

\[
V = \frac{k_{cat}[E][Ab]}{K_m + [Ab]}
\]  

(1)

The main parameters for this are the turnover rate, \( k_{cat} \) (s\(^{-1}\)), and the Michaelis constant, \( K_m \) (\( \mu \)M), the ratio of which \( (k_{cat}/K_m) \) is often used as a measure of enzyme efficiency (Zygmont et al., 1992). \([E]\) and \([Ab]\) are the concentrations of \( \beta \)-lactamase enzyme (sum of both free enzyme and occupied enzyme) and antibiotic, respectively, in the current patch. The reaction rate \( V \) is the amount of antibiotic cleaved (and thus rendered inactive) by \( \beta \)-lactamase per second in the current patch. This results in a decrease in the concentration of active antibiotic \( ([Ab]) \) available to bind to the penicillin-binding proteins (PBP2a) in the bacterial cell membrane (Eq. 2). Therefore, the interaction of \( \beta \)-lactamase enzymes with antibiotics in the environment favours the survival of the bacterial cells producing them.

With regard to the interaction between antibiotic and PBP2a in the bacterial cell membrane, this is a pre-steady state reaction which depends on the concentration of active antibiotic in the patch (Lu et al., 1999). Therefore, the parameters describing this reaction are the acylation rate of PBP2a, \( k_2 \) (s\(^{-1}\)), and the dissociation constant, \( K_d \) (\( \mu \)M). The ratio of these values \( (k_2/K_d) \), or the second-order rate constant, is a convenient measure of the antibiotic efficacy at inhibiting PBP2a function. The maximum proportion of PBP2a that is acylated per second \( (k_o) \) is the apparent first-order rate constant at a given active antibiotic concentration \( ([Ab]) \) is calculated as follows (Chambers et al., 1994):

\[
k_o = \frac{k_2[Ab]}{K_d + [Ab]}
\]  

(2)

There is a variable \( \text{("acylated\_PBP")} \) associated with each bacterial agent that records the proportion of PBP2a that has been acylated. Once the proportion of acylated PBP2a crosses a certain threshold, the cell division is inhibited. This is to represent the natural situation where a critical amount of non-acylated PBP2a molecules are essential for correct cell wall synthesis to take place. The inputted kinetic parameters \( \{k_{cat}, K_m, k_2, K_d\} \) used in this study are listed in Table 1, and were derived from experimental studies in the literature (Fuda et al., 2004; Graves-Woodward and Pratt, 1998; Lu et al., 1999; Zygmont et al., 1992).

3. Results

Fig. 3 shows the simulated growth curve of an MRSA culture in Micro-Gen, with and without the addition of an inhibitory concentration \( (103.1 \mu \text{g}/\text{ml}) \) of cephalothin antibiotic after 3.5 h of incubation. The control culture of MRSA, where no antibiotic is added, displays the characteristic standard growth curve of bacteria grown in nutrient-limited culture conditions. There is an initial lag phase, when the bacteria adapt to the environment and begin synthesising the necessary macromolecules required to survive. The lag phase is represented by a period of time during which the bacterial agent’s nutrient intake rate is increased from an initial low level up to the full rate specified in Table 1. This is followed by an exponential phase of growth, until nutrient deprivation or accumulation of waste products begins to limit growth and the cells enter the stationary phase, followed eventually by the death phase (not shown).

The addition of antibiotic during the exponential phase of growth results in growth inhibition for a limited period of time. The length of time during which bacterial growth is inhibited is important, as it influences the recommended dosage regimen for an antibiotic. It is affected by factors such as the half-life of the antibiotic, and the action of bacterial enzymes, such as \( \beta \)-lactamases, which degrade the antibiotic molecules.

The MIC was calculated from the model for a number of common antibiotics against MRSA, and compared with real-world results. The MICs are estimated from the model in an analogous way to the broth dilution test carried out in the experimental environment: a number of cultures are carried out with different concentrations of antibiotic applied. The minimum concentration of antibiotic that results in complete inhibition of bacterial growth is determined to be the MIC.

In order to quantitatively predict the MICs for antibiotics against a particular strain of bacteria, the \( \beta \)-lactamase production rate per second per bacterial agent was first estimated. This was done by graphing the predicted MIC of penicillin G from the model over a range of \( \beta \)-lactamase production rates (Fig. 4). The production rate that gave an MIC equivalent to the experimentally determined value for penicillin G (in this case Type A MRSA = 72.1 \mu \text{g}/\text{ml}, Type C MRSA = 479.9 \mu \text{g}/\text{ml}) was then used in all further simulations for that strain (Norris et al., 1994). For the Type A \( \beta \)-lactamase-producing strain, the estimated production rate per bacterial agent was \( 1.01 \times 10^{-7} \mu \text{g}/\text{s} \) and for Type C it was estimated to be \( 6.1 \times 10^{-9} \mu \text{g}/\text{s} \).

Fig. 5 contains the predicted MIC values for a number of common antibiotics compared with results from experimental studies published in the scientific literature (Malouin et al., 2003; Norris et al., 1994). The MICs of the antibiotics were calculated for three different types of MRSA bacteria: \( \beta \)-lactamase-producing strains (Type A, Type C), and a \( \beta \)-lactamase-negative strain (see Table 1). All other parameters, including the PBP2a kinetic parameters, were maintained constant across the three types of bacteria.

The results indicate that the model represents a robust tool for predicting the MIC of an antibiotic against various different
strains of bacteria when basic biochemical/kinetic data are available about the antibiotic. A certain degree of variation from the experimental results is to be expected, as the MICs calculated by Norris et al. (1994) represent the geometric mean of several different MRSA strains, whereas the kinetic parameters used for the simulations are specific to one particular strain. Also, it must be noted that the experimental results were derived from cultures grown suspended in liquid medium, whereas Micro-Gen represents cultures growing on a two-dimensional surface. This may contribute to some of the variation between the predicted and experimentally determined results.

The most notable deviation between the predicted and experimentally determined MIC values is for ampicillin against Type A β-lactamase-producing bacteria (53.5 ± 2.3 µg/ml versus 89.3 ± 17.0 µg/ml, respectively). The second-order rate constant for the PBP2α-ampicillin reaction (9.0 M⁻¹ s⁻¹) used in the model was derived from experimental tests by Graves-Woodward and Pratt (1998). However, another study by Fuda et al. (2004) has calculated the value to be 5.0 M⁻¹ s⁻¹ for ampicillin. When the rate constant from Fuda et al. for ampicillin was inputted into the model instead, it outputted a predicted MIC of 96.0 ± 3.9 µg/ml, which is closer to that recorded by Norris (89.3 ± 17.0 µg/ml). This illustrates the point that there may be significant natural variation between different bacterial isolates in different locations. The second-order rate constants calculated by Graves-Woodward and Pratt (1998) were chosen over Fuda et al. (2004) because rate constants for all three antibiotics used in this study were available, while Fuda’s paper did not contain kinetic values for penicillin or cephalothin. Owing to variation in experimental techniques between different studies, it is important to obtain parameter estimates from a single source for consistency.

The effect of varying the catalytic efficiencies (kcat/Km) of Type A and Type C β-lactamases from MRSA on the length of time growth is inhibited by the antibiotics was also investigated. The catalytic efficiencies of the β-lactamase enzymes were varied over the range 10²⁰¹⁰⁻¹⁰⁻¹⁰ M⁻¹ s⁻¹ and plotted against the inhibition times relative to when the standard parameter values are used (Fig. 6). There are significant negative correlations between log β-lactamase efficiency (kcat/Km) and inhibition time for both penicillin G and ampicillin (r = −0.39, P < 0.01), and a weaker but still significant (P < 0.01) correlation with cephalothin inhibition time (Type A: r = −0.30, Type C: r = −0.714). Correlation analyses to calculate the Pearson correlation coefficient (r) and two-tailed significance level (P) were carried out using SPSS statistical analysis software v11.0 (SPSS Inc., Chicago, IL, USA).

However, when correlation analyses are restricted to the smaller, but more biologically realistic, range of catalytic frequencies 10²⁻¹⁰⁻¹⁰⁻¹⁰ M⁻¹ s⁻¹, there is no correlation between inhibition

**Fig. 4.** Predicted Minimum Inhibitory Concentrations (MIC) for penicillin G, ampicillin and cephalothin from Micro-Gen model over a range of different β-lactamase production rates for Type A (A) and Type C (B) β-lactamase enzymes.

**Fig. 5.** Predicted versus experimentally determined geometric mean MIC ± S.E.M. (µg/ml) of penicillin G, ampicillin and cephalothin antibiotics against three different types of MRSA. Experimentally determined MICs are from Norris et al. (1994) and Maloquin et al. (2003). Predicted MICs are derived from triplicate simulations with the geometric mean MIC ± S.E.M. (µg/ml) displayed. (A), Type A β-lactamase-producing MRSA; (C), Type C β-lactamase-producing MRSA; (--), β-lactamase-negative MRSA.
time and log \( k_{cat}/K_M \) for cephalothin over this range \((r = 0.0)\). On the other hand, for penicillin G and ampicillin, there are still significant negative correlations present \((r \approx -0.85, P < 0.01)\). These results agree qualitatively with tests comparing antibiotic administered on its own and in conjunction with a \( \beta \)-lactamase inhibitor sulbactam (Norris et al., 1994). The inhibitor sulbactam competes with antibiotic molecules for binding to \( \beta \)-lactamase, effectively reducing the rate of catalysis of antibiotic (i.e., equivalent to a reduced \( k_{cat}/K_M \)). Norris et al. recorded a significant increase in antibiotic efficacy for penicillin and ampicillin in the presence of \( \beta \)-lactamase inhibitor, whereas cephalothin was unaffected.

The role of PBP2a-binding efficiency in influencing the outcome of treatment was also investigated across the three different types of MRSA strain. The second-order rate constant (a measure of binding efficiency) for the reaction between antibiotic and PBP2a in MRSA was varied over a range of several orders of magnitude for the antibiotics (Fig. 7). There is a strong positive correlation between the binding efficiency of PBP2a and the inhibition time for all the antibiotics against the three types of MRSA \((r < 0.9 \text{ and } P < 0.01)\). However, there is a steeper increase in the inhibition time of cephalothin relative to penicillin G and ampicillin when treating the Type A and Type C \( \beta \)-lactamase-producing strains (up to 4-fold difference in slope of fitted linear regression lines). This difference is much less pronounced in the case of the \( \beta \)-lactamase-negative strain \((<2\text{-fold difference in slope})\).

Once again, these results are in qualitative agreement with experimental findings comparing MRSA bacterial strains (low \( k_2/K_d \) with methicillin-susceptible \( S. aureus \)) (MSSA) strains (high \( k_2/K_d \)) (Miller et al., 2005; Malouin et al., 2003). Miller et al. showed a significant increase in cephalothin efficacy between the MRSA and MSSA strains, which agrees with the results from Fig. 7. On the other hand, the results from Malouin et al. showed no significant difference in the antibiotic efficacies for penicillin/ampicillin when comparing \( \beta \)-lactamase-positive MRSA and MSSA strains, but there was a significant increase when comparing \( \beta \)-lactamase-negative strains. This is also in agreement with Mico-Gen’s predictions from Fig. 7. The model did not show a notable
increase in penicillin/ampicillin efficacy for β-lactamase-positive strains unless \(k_{2}/K_{a}\) was raised to an extremely high level (> 10^3).

4. Discussion

The MIC of an antibiotic is a common high-level measurement for assessing and comparing the efficacies of different antibiotics during the rational development of treatment regimens. The MICs of penicillin G and ampicillin, as well as the MIC of a common first generation cephalosporin antibiotic cephalothin, were predicted by the model (Fig. 5) by inputting the kinetic parameters listed in Table 1. When compared with results from experimental tests of MIC for these antibiotics, the model's predicted values matched closely the real-world results. Further tests will need to be carried out to validate the model with a broader range of β-lactam antibiotics, but these initial results are encouraging. They indicate that a global parameter (MIC) for a bacterial colony can be successfully predicted by inputting parameters at the cellular/molecular level.

The differences between the predicted MICs from the model and the real-world results may be due to subtle differences in the biochemical characteristics of the strains used by Norris et al. (1994), compared to the strains from which the PBP2a and β-lactamase molecular parameters were derived (Fuda et al., 2004; Graves-Woodward and Pratt, 1998; Lu et al., 1999; Zygmunt et al., 1992). It must also be noted that the method for calculating the MICs of antibiotics experimentally is limited in its precision compared to computational predictions.

The experimental MICs derived from Norris et al. were calculated by testing two-fold dilutions of antibiotics ranging from 2 to 2048 μg/ml and calculating geometric means from triplicate tests. This necessarily constrains the precision of the results, which may lead to some of the variation between the predicted MICs and the experimental results. The level of precision of the Micro-Gen model's predicted MICs can be controlled by the user by choosing the amount of different antibiotic concentrations to test. Although greater precision can be obtained from experimental results by similarly testing more concentrations of antibiotic, due to logistical constraints often only two-fold dilution steps are used in the broth dilution process.

In the case of the MIC of ampicillin against Type A β-lactamase, there is some variation in the predicted MIC depending on whether kinetic parameters from Graves-Woodward and Pratt (1998) or Fuda et al. (2004) are used. This illustrates how variation in parameters at the molecular level can have a significant impact on overall treatment response. It is therefore important to obtain reliable, accurate estimates of the pharmacokinetic parameters for the antibiotics and bacterial strains being investigated, in order to make conclusions about the emergent dynamics of the system.

In order to examine the effects of β-lactamase enzymes and PBP2a on the survival of bacteria in the presence of antibiotics, some simulations were carried out using kinetic parameters for penicillin G, ampicillin and cephalothin. The effects of Type A and Type C β-lactamas on the efficacies of penicillin G, ampicillin and cephalothin were examined by exploring the correlation between the length of time during which growth was inhibited by the antibiotics and the catalytic efficiency (\(k_{cat}/K_{m}\)) of the enzyme at hydrolytically cleaving the β-lactam ring (Fig. 6). For both types of β-lactamase, varying the catalytic efficiency against cephalothin by up to three orders of magnitude over the current natural level made little discernible difference to the inhibition time (<1% difference). This could indicate why Type A and Type C β-lactamas have relatively low rates of hydrolysis of cephalothin, compared to penicillin G; bacterial cells expressing more efficient β-lactamase against cephalothin would not have a significant evolutionary fitness advantage.

By contrast, for penicillin G and ampicillin, over the same range of catalytic efficiencies, there is a steep decrease in the inhibition times that strongly correlates with the catalytic efficiency. This suggests there would be positive selective pressure for MRSA strains expressing more catalytically efficient β-lactamas when exposed to penicillin G or ampicillin over extended periods of time.

The other major mechanism of resistance characteristic of MRSA bacteria is the expression of PBP2a protein in the cell membrane, which has decreased binding affinity for β-lactam antibiotics. Fig. 7 displays the results of varying the second-order rate constant (\(k_{2}/K_{a}\)) of the reaction between antibiotic and PBP2a in the cell membrane. As this value is increased, the inhibition times of the antibiotics also increase, as expected because the damage they inflict on the bacterial cell is proportional to their ability to bind to and inhibit PBP2a function.

However, the slope of the curve for cephalothin is significantly steeper than either penicillin G or ampicillin when strains of MRSA expressing either Type A or Type C β-lactamase are tested. This indicates that the PBP2a status of a bacterial cell may have a more marked impact on the clinical outcome of cephalothin treatment than penicillin G or ampicillin treatment, in β-lactamase-expressing strains. This agrees with experimental studies which have shown that the MICs for penicillin/ampicillin are the same in either MRSA (PBP2a-positive, low \(k_{2}/K_{a}\)) or MSSA (PBP2a negative, high \(k_{2}/K_{a}\)) strains of β-lactamase-producing bacteria, whereas there is a marked difference recorded experimentally (>100-fold) depending on the PBP2a status for cephalothin (Miller et al., 2005).

When a β-lactamase-negative strain of MRSA is tested, on the other hand, the efficacies of penicillin G and ampicillin are more strongly influenced by their PBP2a-binding affinities. This is because the β-lactamase enzyme is not present to limit the efficacies of the antibiotics. As the β-lactamase status and production rate can vary considerably across different strains of MRSA, it is important to be able to predict quantitatively how these differences will affect the treatment response in a particular infection.

The results from these investigations indicate that the Micro-Gen Bacterial Simulator is a useful tool for quantitative analysis of antibiotic-resistance strategies in MRSA bacteria. It provides a means for integrating information obtained at the molecular level into a model of overall population dynamics. As each bacterial cell is represented by a unique software agent, this allows the inherent heterogeneity of the bacterial colonies to be modelled. This is the main advantage of the agent-based modelling approach over other methods, as many mathematical models assume population-averaged parameters for bacteria. It is also a challenge, as the reliability of the model depends on the availability of good experimental data about individual bacterial cells.

Although advances in recent years have increased our knowledge about the cell biology of individual bacteria, most experimental data is still derived from population-level studies. It is important to obtain data at the cellular level in order to derive relevant insights from the agent-based approach. The use of population-averaged parameters would produce results similar to high-level mathematical models. In that case, due to the extra computational constraints associated with a low-level agent-based model, it would not represent an advantage over existing techniques. The power of the agent-based approach exists in being able to vary parameters for individual bacteria such as the growth rate or antibiotic susceptibility while taking into account spatial heterogeneity in the environment.
The sensitivity of the model has been tested with respect to environmental parameters such as the rate of diffusion and the bacterial population size (unpublished results). The model is robust to variations in these parameters that may be expected to occur in normal laboratory-controlled growing conditions. However, the effects of more extreme variations in these parameters will be the subject of a future study to explore the impact of changing environmental conditions on treatment outcome, and how they may contribute to some of the differences in antibiotic efficacy often observed between the in vitro and the in vivo situation.

Traditional methods of measuring antibiotic efficacy such as the MIC are insufficient for understanding the complex dynamics that lead to the rapid development and spread of antibiotic resistance within bacterial populations. However, the ability to investigate the relationship between individual molecular components of the system and the overall treatment outcome can lead to a better understanding of how to optimise antibiotic performance and predict treatment outcome. Micro-Gen can also be used to indicate evolutionary pathways or dead ends that may exist for bacteria in response to antibiotic exposure.

The model is highly adaptable and capable of being scaled up from lightweight portable devices to high-performance parallel computing machines. The user can input parameters applicable to different species of bacteria by modifying low-level cellular attributes such as size, growth rate, motility, etc. However, as shown here, it can also be adapted to incorporate detailed representations of specific antibiotic-resistance strategies such as those employed by MRSA.

Future work will include using the model to investigate the system dynamics of combinatory therapy with multiple classes of antibiotic. It can also be used to test hypothetical scenarios by varying the parameters of existing antibiotics to explore how potential novel compounds might act. It is a useful tool for the rapid screening of drug compounds against a diverse range of S. aureus strains in simulated culture conditions. The agent-based approach is also suitable for modelling the evolution of antibiotic resistance over time by incorporating genetic components into the bacterial agents. This would allow both the temporal and spatial dynamics of antibiotic-resistance development to be examined.

Another important future aim of the Micro-Gen project is to model β-lactamase-dependent pro-drug delivery systems. Under these systems, a substrate-like pro-drug molecule containing the β-lactam ring structure undergoes therapeutic activation catalysed by β-lactamases to achieve selective release of a cytotoxic anti-microbial agent (Bush et al., 2004; Stone et al., 2004). Micro-Gen would be a useful tool to examine the dynamics of this system of activation and assess its therapeutic potential from a theoretical standpoint.

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