A COMPUTATIONAL MODELLING APPROACH TO EXPLORE THE ANTI-MICROBIAL PRO-DRUG DELIVERY SYSTEM

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Keywords: Agent-based model, Antibiotic resistance, Pro-drug, Bacteria, Simulation.

Abstract: This article documents simulations using an agent-based modelling approach to analyse the system dynamics of the β-lactamase-dependent therapeutic activation pro-drug delivery system, a novel approach for achieving selective release of anti-microbial drugs for treating antibiotic-resistant bacteria. It is thought that this strategy could be a promising approach for treating β-lactamase over-expressing strains of bacteria that are resistant to traditional β-lactam antibiotics such as penicillin. Test simulations were carried out to investigate the pro-drug system from a theoretical standpoint and assess the effects of key parameters such as half-life, diffusion rate and reaction kinetics on the system behaviour. It is important to obtain a thorough understanding of the complex interplay between the various components involved in the pro-drug delivery system to be able to interpret results from laboratory testing, and ultimately, from the clinical setting. The agent-based model described here represents an important stepping stone in connecting the theoretical and practical understanding of the system as a whole.

1 INTRODUCTION

Our lab is inovolved in developing an agent-based model, called Micro-Gen, which simulates the growth of bacterial cells in culture and their interactions with anti-microbial drug molecules (Murphy et al., 2007; Murphy et al., 2008; Murphy et al., 2009). The program uses an agent-based modelling approach whereby the individual bacterial cells are represented by unique software agents that are capable of flexible, autonomous action within a simulated environment (Jennings et al., 1998). The agent-based approach means that the system as a whole can exhibit a complex behaviour that is more than the sum of its constituent parts. This approach allows the unique dynamics within a bacterial colony to be simulated by taking into account the temporal and spatial heterogeneities within the population.

So far, Micro-Gen has been used in previous studies to examine the role of various low-level cellular parameters in the response of bacterial populations to antibiotic treatment (Murphy et al., 2009). Studies showed that it could accurately predict the minimum inhibitory concentrations (MIC, a simple laboratory measure of antibiotic efficacy) for various common β-lactam antibiotics, including penicillin G and cephalothin, against methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria (Murphy et al., 2008). However, another strength of the model exists in being able to examine new approaches for treating antibiotic-resistant bacteria and give insight into potential novel drug treatment strategies. This can aid in rational drug design by allowing a greater understanding of the underlying mechanistic principals determining response to treatment.

In the present study, the existing model has been adapted to explore a novel approach to treating antibiotic-resistant bacteria called the β-lactamase-dependent pro-drug delivery system (Smyth et al., 2000). This approach involves administering a substrate-like pro-drug molecule that contains a β-lactam ring structure (Rautio et al., 2008). Therapeutic activation of the pro-drug occurs when its β-lactam ring structure is cleaved by β-lactamase enzymes released from the bacterial cells. This cleavage results in the selective release of a molecule with anti-

T. Murphy J., Walshe R. and Devocelle M..
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DOI: 10.5220/0003154903010308
In *Proceedings of the International Conference on Bioinformatics Models, Methods and Algorithms* (BIOINFORMATICS-2011), pages 301-308
ISBN: 978-989-8425-36-2

microbial properties that kills or inhibits the growth of the bacterial cells (Stone et al., 2004; Bush et al., 2004).

This is considered a promising therapeutic approach because many bacterial species (e.g. *Staphylococcus aureus*) have evolved to produce β-lactamase enzymes in response to prolonged clinical exposure to β-lactam antibiotics such as penicillin G (Abraham and Chain, 1940). The bacterial cells produce β-lactamase enzymes as a defence mechanism because the enzymes cleave the β-lactam ring structure present in the antibiotic molecules, rendering them inactive (Fisher et al., 2005). In the USA, it is estimated that greater than 95% of all *S. aureus* bacterial isolates possess resistance to penicillin, due to the expression of β-lactamase (Levy and Marshall, 2004; Neu, 1992). Therefore, designing pro-drugs that specifically target these bacteria would introduce an evolutionary selective pressure contrary to that of existing β-lactam antibiotics.

2 MODEL OVERVIEW

The simulations described here were carried out using an adapted version of the Micro-Gen Bacterial Simulator. Previous versions of Micro-Gen were used to model traditional β-lactam antibiotics. But here we describe initial efforts to model a new class of drugs called enzyme-catalysed therapeutic activation (ECTA) pro-drugs. A detailed description of the underlying program structure along with an analysis of the mechanistic basis for its output has been previously published (Murphy et al., 2008; Murphy et al., 2009).

The individual bacterial cells are represented by software agents that store physical traits such as their energy state or amount of antibiotic damage. The agents also have local behavioural rules associated with them that dictate their actions during the simulation. The behaviour of the colony as a whole is an emergent property of the individual agent interactions. The environment of the simulations is represented by a discrete, two-dimensional grid containing diffusible elements such as nutrients, β-lactamase enzymes and anti-microbial drug compounds (fig. 1). The movement of these molecules is dictated by a discrete implementation of Fick's First Law of diffusion (Ginovart et al., 2002).

The key interaction of the model that determines the response to pro-drug treatment is the reaction between the β-lactamase enzyme and the pro-drug. This reaction is the activation step that triggers the release of an active drug compound (fig. 2). The success of

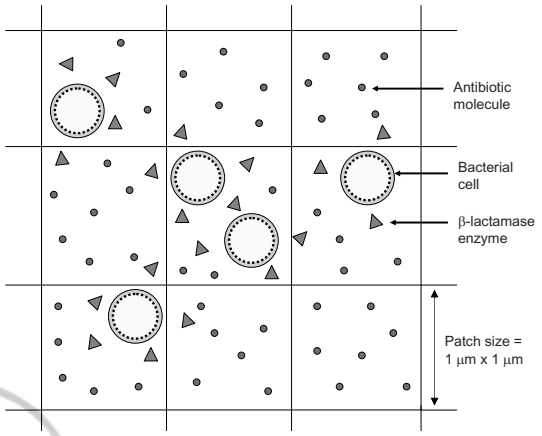

Figure 1: Diagram of discrete, two-dimensional simulation environment and key components of simulation (bacterial cells, antibiotic molecules, and β-lactamase enzymes). Each grid element represents 1 μm^2 area of environment.

the pro-drug approach requires the rapid and specific release of the active drug compound in the vicinity of the bacterial cells. The model contains a quantitative representation of this reaction based on Michaelis-Menten kinetic theory. The equation for calculating the reaction rate (V) is as follows:

$$V = \frac{k_{cat}[E]_t[Ab]}{K_M + [Ab]} \quad (1)$$

The key parameters are the turnover rate, k_{cat}, and the Michaelis constant, K_M. The ratio k_{cat}/K_M is often used as a measure of enzyme efficiency (Zygmunt et al., 1992) These parameters can be calculated from biochemical studies in the laboratory and are specific to the type of drug used and strain of bacteria. $[E]_t$ and $[Ab]$ are the concentrations of β-lactamase enzyme and antibiotic in the local grid element respectively.

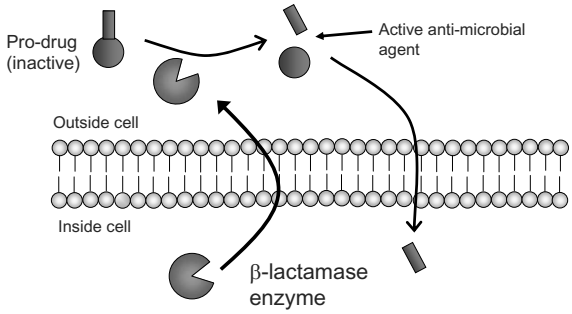

Figure 2: Diagram of activation of pro-drug molecule triggered by cleavage of its β-lactam ring structure by β-lactamase enzyme. The active component of the pro-drug can then enter the cell and inhibit growth/destroy the cell (depending on the mode of action of the drug).

For the interaction between the activated drug compound and its target molecule in the bacterial cell a model of pre-steady state reaction kinetics is used. The specific target molecule depends on the the type of anti-microbial drug compound used. It must be noted that the activated drug is assumed to lack the β-lactam ring structure, and thus it is not subject to cleavage and re-inactivation by β-lactamases in the simulation.

The model is sufficiently generalised so that it is not specific to a particular drug/target combination. The key parameters that are required are k_2, the rate of inactivation of the target molecule, and K_d, the dissociation constant. The ratio of these values (k_2/K_d), or the second order rate constant, is a convenient measure of the drug's efficacy at inhibiting the target molecule's function. The proportion of target molecule that is inactivated per second, k_a (the apparent first order rate constant), at a given drug concentration is calculated as a function of these parameters (equation 2):

$$k_a = \frac{k_2[Ab]}{K_d + [Ab]} \quad (2)$$

2.1 Paramaters for Test Simulations

The speed and efficiency of activation of the pro-drug is an important factor for determining the efficacy of a pro-drug delivery system. A number of simulations were carried out to examine the effects of several different parameters on the activation of the pro-drug and inhibition of bacterial growth by the activated product. The same cellular parameters for representing β-lactamase-producing MRSA bacteria that were used in previously published investigations were applied here (Table 1) (Murphy et al., 2008; Murphy et al., 2009). A hypothetical penicillin-based pro-drug was simulated, i.e. the kinetic parameter values (k_{cat}/K_M) for penicillin G were used to define the interaction between β-lactamase and the pro-drug molecule. The kinetic values for penicillin G were chosen because this represents a situation where the β-lactamase enzymes have a high catalytic efficiency versus the sample pro-drug. This represents an optimal situation in order to assess the potential of this approach.

The active drug compound that arises from cleavage of the simulated penicillin-based pro-drug has kinetic parameters (k_2/K_d) which determine the rate of binding to the bacterial cell (Table 1).

Table 1: Inputted parameter values for simulations of pro-drug interactions with β-lactamase-producing *S. aureus* bacteria in Micro-Gen model. *b.u.* = biomass units; *loop* = program loop (∼2 s in real time).

Parameters (units)	**Input Value**
Environment:	
Patch size (*b.u.*)	20000
Patch nutrient level (*b.u.*)	80000
Diffusion co-efficient	0.1
Bacterial agents:	
Generation time (*min*)	29
Threshold for division (*b.u.*)	10000
Nutrient intake ($b.u.\ loop^{-1}$)	10.0
Survival cost ($b.u.\ loop^{-1}$)	0.2
Stationary phase rel. metabolic rate	0.2
Lag phase length (*min*)	63
β-lactamase:	
Production rate ($\mu M\ s^{-1}$):	3.28×10^{-7}
Production cost (*b.u.*)	0.1
Molecular weight (*Da*)	30000
Half-life (*s*)	53640
k_{cat} (s^{-1}):	171.0
K_M (μM):	51.1
Pro-drug:	
Half-life (*s*)	2520
k_2 (s^{-1}):	0.185
K_d (μM):	1540

3 RESULTS AND DISCUSSION

3.1 Introduction

In order to test the ability of the model to reproduce real world behaviour of pro-drug compounds a couple of case studies were carried out previously. These case studies involved taking kinetic parameters for two pro-drug compounds from the literature, called NB2001 and NB2030, and running simulations in order to test the output of the model (Li et al., 2002; Stone et al., 2004). A detailed description of these tests is included in a previous publication along with analysis and discussion of the results (Murphy et al., 2010). A comparison between the predicted MICs for NB2001 and NB2030 and the experimentally-determined values are included in figure 3. The predicted values matched closely the experimental results, which indicated a sound theoretical basis for the model.

However, the power of any modelling approach does not exist in making predictions, but rather in providing a basis for a thorough investigation of the dy-

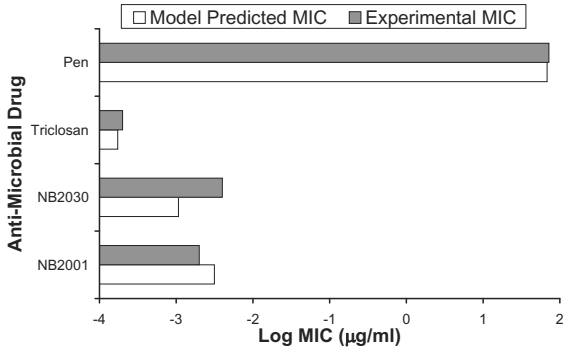

Figure 3: Predicted Minimum Inhibitory Concentrations (MICs) for two pro-drug candidates, NB2001 and NB2030, described in literature. Predicted MICs for Penicillin G (a β-lactam antibiotic) and Triclosan (an inhibitor of the bacterial fatty acid synthesis cycle, and the active component of NB2001 and NB2030 (Slater-Radosti et al., 2001)) are also included for reference.

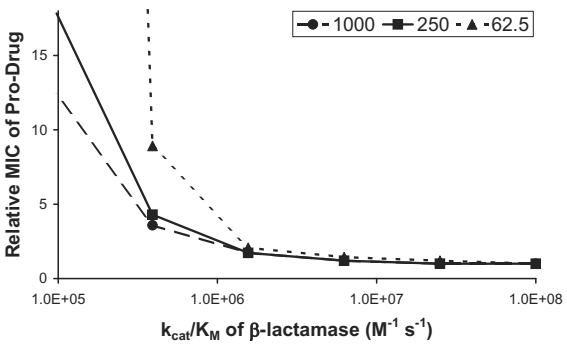

Figure 4: Effect of catalytic efficiency (k_{cat}/K_M) of β-lactamase enzyme on minimum inhibitory concentration (MIC) of pro-drug. Three different pro-drugs are graphed which differ by the rate of binding of their activated antimicrobial agent to the bacterial cell (k_2/K_d of activated antimicrobial agent: 62.5, 250 and 1000 M^{-1} s^{-1}).

namics of the system. With that in mind, the focus of this article is to extend our research to encompass a more theoretical exploration of the pro-drug system in order to identify the factors that influence the output. By developing a more holistic understanding of the pro-drug system, a more rational approach to designing pro-drug candidates can be developed.

3.2 Effect of Kinetic Parameters on Drug Efficacy

Figure 4 shows the results of tests investigating the effect of the kinetic parameters on the MIC of the penicillin-based pro-drug. A lower MIC means a lower concentration of pro-drug is required in order to inhibit the bacterial growth. Three hypothetical variations of the pro-drug were investigated, which differed by the rate of binding of the activated antimicrobial agent to its target in the bacterial cell (k_2/K_d = 62.5, 250, and 1000 M^{-1} s^{-1}). The catalytic efficiency of the β-lactamase enzyme (k_{cat}/K_M) at cleaving and activating the pro-drug was assessed by varying over a range of 10^5 - 10^8 for each pro-drug variant. As would be expected, higher values for the catalytic efficiency result in a lower MIC for the pro-drug.

Figure 4 shows that for these pro-drugs the predicted MIC decreases with increasing catalytic efficiency of the β-lactamase enzyme. The pattern is the reverse of the trend seen in traditional β-lactam antibiotics (Murphy et al., 2008). This is one of the reasons why there is interest in the β-lactamase-dependent pro-drug delivery system. It would be expected that administration of β-lactamase-dependent pro-drugs could lead to evolutionary selective pressure opposed to that exerted by β-lactam antibiotics.

The dynamics between the negative selective pressure from pro-drugs and positive selective pressure from β-lactam antibiotics would be an important factor to consider when assessing the possible evolution of drug resistance in bacteria in response to these two different therapeutic strategies. However, the complex interplay of biophysical, pharmacokinetic, pharmacological and epidemiological factors which would contribute to this are beyond the scope of this study. Nevertheless, this modelling approach is useful for developing theories about how molecular parameters may contribute to the observed dynamics of the system.

3.3 Effect of β-lactamase Production Rate on Pro-drug Activation

It is clear that the β-lactamase production rate of the bacterial cells is an important parameter to be considered when investigating the β-lactamase-dependent pro-drug delivery system. The production rate can vary considerably between different bacterial strains, and this must be factored in when assessing the usefulness of this drug delivery system. Figure 5 shows the growth dynamics of a bacterial population when exposed to a penicillin-based pro-drug (1.8 μg/ml), with the β-lactamase production rate varied between 10^{-7} - 10^{-5} μM s^{-1} $agent^{-1}$ For reference, the β-lactamase production rate for naturally occurring Type A MRSA under these simulation conditions was estimated to be 3.28×10^{-7} μM s^{-1} $agent^{-1}$ (Murphy et al., 2009).

The results confirm the important role that the β-lactamase production rate has on the efficacy of the β-lactamase dependent pro-drug delivery system. For these simulations, it is assumed that there is no

contamination or spontaneous activation of the active anti-microbial agent apart from activation by β-lactamase. In real life, there may be some contamination with active compound that would lead to positive results even when treating β-lactamase-negative strains of bacteria. However, this ambiguity can lead to problems when assessing the true effectiveness of the approach.

The active drug concentration threshold required for inhibition of growth is approximately 0.8 μg/ml. This threshold is determined by the minimum inhibitory concentration of the activated antimicrobial agent. It is noteworthy however, that increasing the β-lactamase production rate from 10^{-6} to 10^{-5} μM s^{-1} $agent^{-1}$ (fig. 5B - C) does not result in a corresponding increase in the length of time bacterial growth is inhibited for. The inhibition time seems to be limited by the half-life of the drug in this case (see fig. 6).

3.4 Effect of Half-life on Pro-drug Activation

One of the most important parameters that limit the efficacy of both traditional antibiotics and novel drug candidates, such as pro-drugs, is the half-life of the molecule. However, the impact of this parameter can vary substantially depending on the type of antibiotic used (Murphy et al., 2009). The half-life of a molecule can vary dramatically depending on local environmental conditions, such as pH or temperature variations. It is important to determine its influence in order to attempt to predict treatment success.

For this reason, computational analyses were carried out to predict the impact of this parameter on the pro-drug delivery system (fig. 6). The half-life of the simulated pro-drug was varied between 16 minutes and 2.8 hours, and the growth curve of the bacterial population plotted along with the concentration curve of activated anti-microbial agent.

When the half-life is $\leq$16 minutes, the concentration of activated anti-microbial agent never exceeds the threshold required for growth inhibition, 0.8 μg/m (fig. 6A). Therefore, the bacterial population follows the standard growth curve, eventually entering the stationary phase due to nutrient limitations. However, when the half-life of the pro-drug is increased to 1.4 hours or greater, then the required concentration of active drug compound is reached and inhibition of growth occurred.

The half-life of a pro-drug is therefore a very important parameter when determining its efficacy against β-lactamase-producing *S. aureus* bacteria. This system is particulary sensitive to the half-life parameter because of the time delay between administration of the pro-drug and activation of sufficient quantities of active agent to inhibit growth.

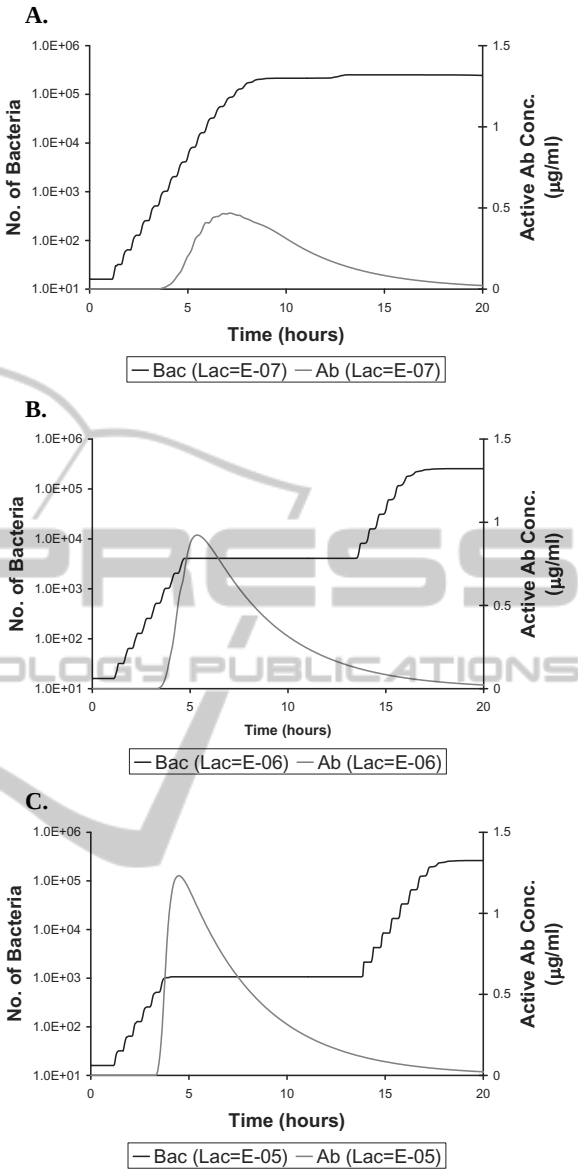

Figure 5: Effect of varying the β-lactamase production rate on the inhibition of β-lactamase-producing *S. aureus* bacterial growth by the penicillin-based pro-drug. The graphs display the simulated log bacterial growth curve along with the concentration of activated drug molecules (μg/ml). 1.8 μg/ml of pro-drug added at time = 3.3 hours. β-lactamase production rates: **A.** 10^{-7} μM s^{-1} $agent^{-1}$; **B.** 10^{-6} μM s^{-1} $agent^{-1}$; **C.** 10^{-5} μM s^{-1} $agent^{-1}$.

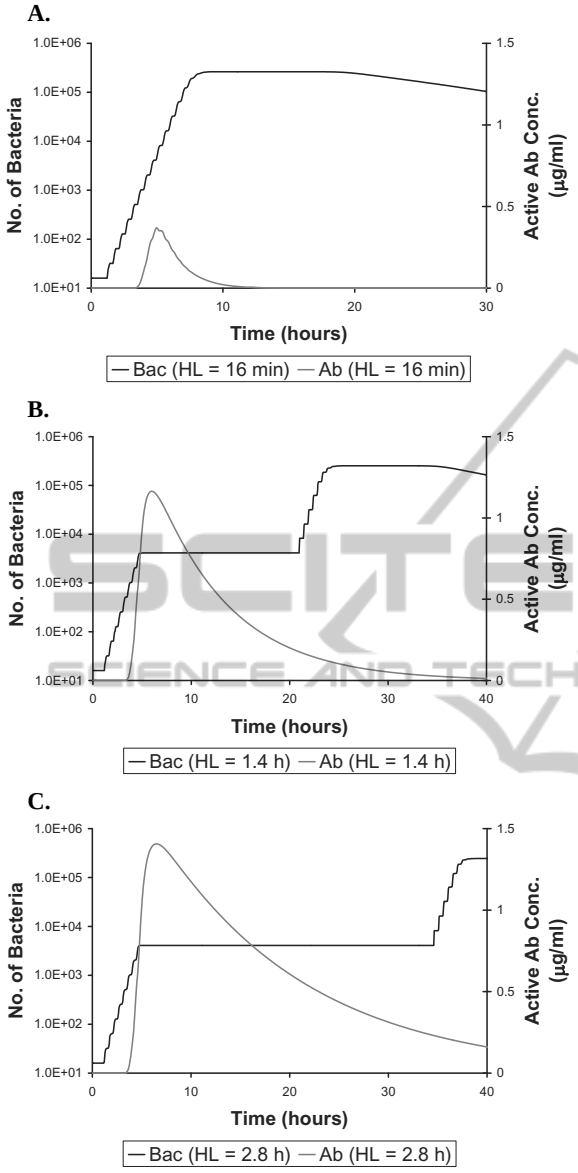

Figure 6: Effect of varying the half-life parameter for the penicillin-based pro-drug on the inhibition of β-lactamase-producing *S. aureus* bacterial growth. The graphs display the simulated log bacterial growth curve along with the concentration of activated drug molecules (μg/ml). 1.8 μg/ml pro-drug added at time = 3.3 hours. **A.** Half-life = 16 *min*; **B.** Half-life = 1.4 *h*; **C.** Half-life = 2.8 *h*.

3.5 Effect of Diffusion Rate on Pro-Drug Activation

The impact of diffusion on the activity and dynamics of the pro-drug delivery system was also investigated. This is an important parameter to assess because the pro-drug delivery system depends on the targeted release of active anti-microbial agents in the close vicinity of bacterial cells. The implementation of an algorithm based on Fick's First Law of diffusion allows some insights to be obtained on the role of diffusion dynamics in the system. The agent-based approach allows us to explicitly take into account spatial heterogeneity in the environmental conditions (e.g. between the inside and outside of the colony) which is important when considering features such as diffusion.

Figure 7 shows the impact of varying the rate of diffusion in the environment on the activation and efficacy of a penicillin-based pro-drug. The rate of diffusion was varied by modifying the user-defined diffusion coefficient (*D*) for Fick's First Law of diffusion. This is a system-level parameter that alters the rates of diffusion of all the molecules (pro-drug, active drug, β-lactamase and nutrients) in the environment. Higher values correspond to a more fluid/dynamic environment whereas lower values result in a more viscous/inert simulated environment. The results from these tests indicate that the rate of diffusion has an important influence on the availability of activated pro-drug in the vicinity of the bacterial cells as measured by the height of the peak in the concentration of activated anti-microbial agent (fig. 7).

When the diffusion rate was lower (fig. 7A), the concentration of activated anti-microbial agents in the local vicinity of the bacterial cells increased more rapidly. This may be explained by the fact that with lower diffusion rates, the activated drug molecules would not be dispersed as quickly from the vicinity of the bacterial cells by natural diffusion processes (fig. 8A).

The pro-drug delivery system results in higher concentrations of activated drug molecules in the direct vicinity of the bacterial cells. The system is therefore sensitive to any forces, such as diffusion or flow forces, that may result in dispersal of the activated compounds. It is important, therefore, to take this into account when designing pro-drugs and try to take measures to minimize this such as, for example, designing molecules that have a greater binding affinity or are electrostatically attracted to the bacterial cells.

This problem is not so evident with traditional antibiotic approaches because they usually involve the administration of relatively high doses of active anti-microbial agent that are not specifically targeted to the local vicinity of the bacterial cells. In fact for some types of β-lactam antibiotic, such as penicillin G, increasing the diffusion rate was predicted to increase antibiotic efficacy (Murphy et al., 2009). This could be due to the fact that higher rates of diffusion results in dispersal of β-lactamase-inactivated penicillin G in the vicinity of the bacterial cells and replacement by active penicillin G from elsewhere in the environment

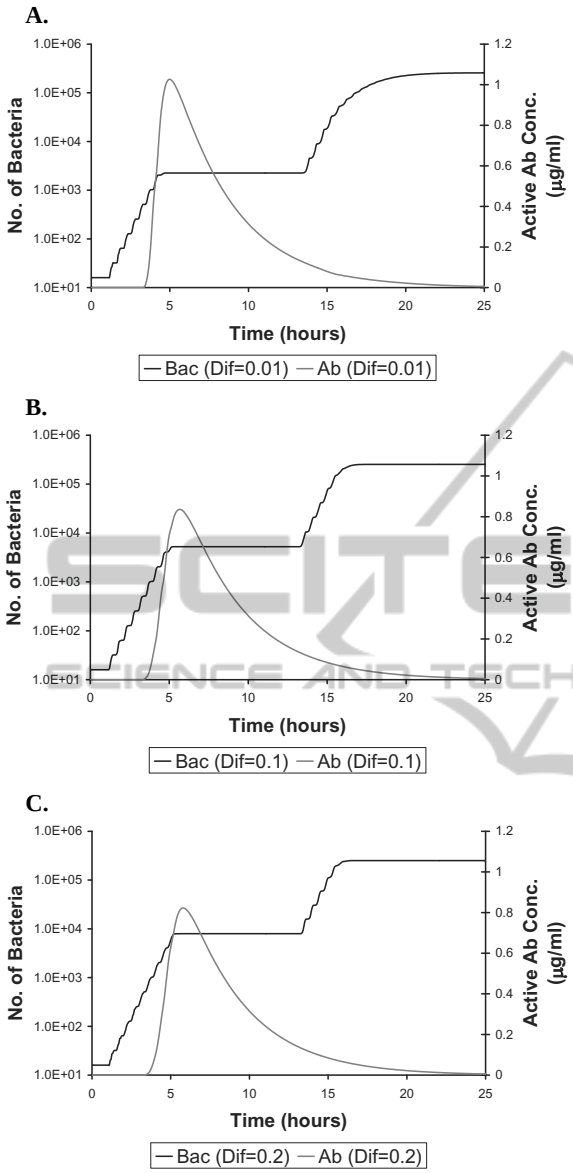

Figure 7: Effect of varying the user-defined diffusion parameter (D) for Fick's First Law of diffusion on the inhibition of β-lactamase-producing *S. aureus* bacterial growth by the penicillin-based pro-drug. The graphs display the simulated log bacterial growth curve along with the concentration of activated drug molecules (μg/ml). 1.8 μg/ml of pro-drug added at time = 3.3 hours. **A.** $D = 0.001$; **B.** $D = 0.01$; **C.** $D = 0.1$.

- the reverse situation to the pro-drug system (fig. 8B).

4 CONCLUSIONS

This paper documents tests to analyse the system dynamics of the β-lactamase-dependent therapeutic activation pro-drug delivery system, a novel approach for achieving β-lactamase-mediated selective release of antimicrobial agents. It is thought that this strategy might be a promising approach for treating β-lactamase over-expressing strains of bacteria that are resistant to traditional β-lactam antibiotics. The initial results are promising and illustrate the power of the computational approach for exploring the mechanisms of action of novel drug compounds. In conjunction with laboratory testing, great insights can be made into the complex interplay of the different components in the pro-drug delivery system using an agent-based modelling approach. Initial work has already been carried out on data from real-life pro-drug candidates in order to compare the model output to experimental results (Murphy et al., 2010). However, the power of the model exists in being able to explore hypothetical scenarios and compounds in order to gain an integrated understanding of the unique dynamics of the pro-drug delivery system. By using different modelling approaches to inform decisions in the rational drug design process it is possible to optimize the effectiveness of this technique so as to offer a viable alternative treatment strategy for microbial infectious diseases.

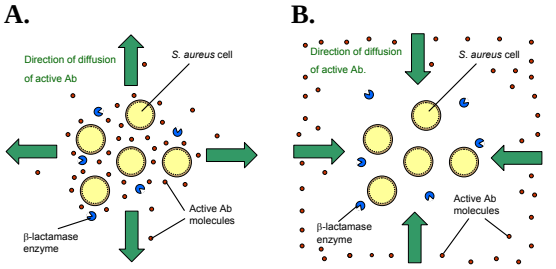

Figure 8: Comparison of the local diffusion gradients of active antimicrobial agents in the local environment of β-lactamase producing *S. aureus* cells for pro-drug (**A**) and traditional β-lactam antibiotics (**B**). **A.** When administered in pro-drug form the active antimicrobial agent concentration is highest in the vicinity of the bacterial cells due to β-lactamase-mediated activation. **B.** For traditional β-lactam antibiotics the concentration of active agent is depleted in the vicinity of the bacterial cells due to inactivation by the β-lactamases and uptake by the cells.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution of Mathieu Joubert, Grainne Kerr, Chris Pender, Marian Duggan and Ronan Winters for the development of the original BAIT software tool. High performance computing resources were provided by the Centre for Scientific Computing and Complex Sys-

tems Modelling (SCI-SYM) in Dublin City University, and the Irish Centre for High End Computing (ICHEC).

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