Probing the interplay of microbes and medicine in a hospital setting using an individual-based model

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1 Preface

The work has received funding from the Olvi foundation and Betty Väänänen foundation. Their support enabled me to invest in more powerful computing equipment and to focus on the thesis more efficiently. I wish to thank my supervisor Matti Jalasvuori for his supportive role and creative ideas. I would also like to thank Hanna Kokko for her feedback on the model.

University of Jyväskylä **Abstract of Master´s Thesis**

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Abstract:

The objective of this project was to develop a simulation environment with a graphical user interface for the study of nosocomial infections. The software allows for the tuning of nearly 40 different hospital parameters and for the export of results in spreadsheet form. The individual-based model focuses on interactions between health-care workers and patients. Pathogens are spread between patients by the workers and the ensuing infections are modeled mathematically. Pathogen mutation to antibiotic-resistant strains is possible and the pathogens may outcompete one another. Phage therapy, the utilization of bacteriophages in treating infections, is included alongside antibiotics as a treatment option. The model indicates that simultaneous use of phage therapy and antibiotics has clear advantage to exclusive use: when simultaneous use is allowed the prevalence of susceptible bacteria decreases by 29% and that of antibiotic resistant bacteria by 10% on the hospital population level. A sensitivity analysis reveals that treatment probability, a parameter describing the likelihood of an infection being noticed, is highly influential in determining average duration of stay: 10% treatment probability results in average duration of stay of 16 days, whereas 100% certainty results in average duration of stay of 7 days. The values in-between follow exponential law. Hand-washing compliance is observed to be strongly and reversely correlated with the prevalence of pathogens. However, full compliance does not entirely eradicate the bacteria, nor does zero-compliance result in total saturation of the population. These limits are due to constant in- and outflux of patients, some incoming patients being colonized prior to arrival. Full saturation of the patient population does not occur when the rate of transfer is low enough to allow patients to leave the hospital before becoming infected. Significance of the results is highly dependent on parameter values, which are often very speculative. The model should therefore be primarily used to study dependencies and sensitivities of parameters. The work lays foundation for the development of more general vector-mediated simulation engines in the future.

Keywords: antibiotic resistance, phage therapy, individual based model, simulation

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Tiivistelmä:

Tämän tutkielman tavoitteena oli kehittää graafisen käyttöliittymän omaava monipuolinen simulaatioympäristö, jolla voidaan mallintaa bakteerien leviämistä sairaalaympäristössä. Ohjelma mahdollistaa noin neljänkymmenen sairaalaparametrin asettamisen ja tulosten purkamisen taulukkomuotoon. Kyseisessä yksilöpohjaisessa mallissa keskitytään sairaalatyöntekijöiden ja potilaiden välisiin vuorovaikutuksiin. Työntekijät toimivat vektoreina, levittäen bakteeri-infektioita potilaiden välillä. Bakteerimallinnus yksittäisen potilaan sisällä on toteutettu matemaattisesti. Bakteerit voivat kilpailla keskenään ja muuntua antibiooteille vastustuskykyisiksi; tällöin voidaan hyödyntää faagiterapiaa, jossa infektioita hoidetaan bakteriofaageilla. Malli osoittaa, että antibioottien ja faagiterapian yhtäaikainen käyttö on huomattavasti tehokkaampaa kuin eriaikainen käyttö: yhtäaikaisessa käytössä sensitiivisten bakteereiden kokonaismäärä sairaalassa laski 29% ja antibioottiresistenttien bakteereiden määrä 10%. Sensitiivisyysanalyysissa selvisi, että esimerkiksi hoitotodennäköisyysparametrilla on suuri, eksponentiaalinen vaikutus keskimääräiseen sairaalassaoloaikaan: 10% hoitotodennäköisyys johti 16 päivän keskipituuteen, kun 100% todennäköisyys johti 7 päivän mittaiseen keskipituuteen. Työntekijöiden hygieniakäytännöt vaikuttavat vahvasti bakteerien kokonaismäärään sairaalassa. Täydet hygienatoimet eivät kuitenkaan riitä koko sairaalan bakteerikannan tuhoamiseen, eivätkä olemattomat hygienatoimet johda jokaisen potilaan infektioon. Tulosten merkityksellisyys on vahvasti riippuvaista parametrien arvoista, joista osa on laajalti spekulatiivisia. Mallia tulisi siis lähinnä käyttää riippuvaisuuksien etsimiseen ja herkkyyksien arvioimiseen. Työ tarjoaa pohjan vektorivälitteisten ja yksilöpohjaisten simulaatiomallien kehittämiseen tulevaisuudessa.

Avainsanat: antibioottiresistenssi, faagiterapia, yksilöpohjainen malli, simulaatio

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2 Abbreviations

HCW: health care worker

HGT: horizontal gene transfer

PT: phage therapy

R_A: antibiotic resistant bacterium

Rd: growth rate

R_P: phage therapy resistant bacterium

S: susceptible bacterium

3 Introduction

Modeling the spread of infectious diseases has been central to understanding patterns in local outbreaks and global pandemics (Siettos and Russo, 2013). Models may also provide insight into responding to such diseases via medicinal methods. Real-world human experiments involving experimental drugs are often expensive, time-consuming and under strong ethical scrutiny. The effects of drugs may also include distinct population-level properties, unforeseen by studies done on the individual level (Koopman and Longini, 1994).

In this thesis I present a modeling environment for investigating the spread of infectious pathogens within a hospital setting. The model is designed as a versatile tool with applications ranging from simple single-pathogen dispersal simulations to more complex systems encompassing the dynamics of antimicrobial resistance. I begin by describing the basics of antimicrobial resistance and then proceed to explore how modeling can be utilized in planning suitable counter-practices.

3.1 Antibiotic resistance and phage therapy

The development of resistance towards fitness-reducing agents often occurs among organisms that thrive in large numbers and have short generation lengths. The evolution of resistance has been observed over a range of lineages, including insects (Mallet et al., 1990), rodents (Ishizuka et al., 2008) and bacteria. The latter represents one of the major concerns in modern healthcare (Spellberg et al., 2013).

3.1.1 Antibiotic resistance

Antibiotics are compounds that kill or inhibit the growth of microorganisms or, from an evolutionary viewpoint, reduce their fitness. Although most commercial antibiotics are produced synthetically (Nussbaum et al., 2006), antibiotics have been abundant in nature for millions of years (Siettos and Russo, 2013; Spellberg et al., 2013). Not surprisingly, resistance towards antibiotics has been prevalent for an equal amount of time. The first signs of resistance to commercial antibiotics were discovered even before penicillin was widely in use (Koopman and Longini, 1994; Abraham and Chain, 1940). Soil bacteria are known to harbor resistance (Riesenfeld et al., 2004) and recently a bacterial strain isolated

in a cave for 4 million years was found to be resistant to 14 different “commercial” antibiotics (Bhullar et al., 2012).

The use of antibiotics generates selection gradients, in which resistant mutants are favored over the wild types. Selection is most efficient in drug concentrations that settle within the “mutant selection window”, whose lower boundary marks the minimum inhibitory concentration and upper boundary the mutant prevention concentration (Drlica, 2003). Resistance may rise due to a random mutation in the bacterial genome or, more commonly, due to horizontal gene transfer (HGT) (Bennett, 2008). The workhorses of HGT, plasmids, are circular DNA elements that depend upon the cellular machinery of their host bacterium to replicate. Plasmids can survive in the extracellular medium as passive elements and occasionally become absorbed by bacteria by the process of transformation. Many plasmids grant their host antibiotic resistance and also the ability to perform inter-bacterial HGT. Such direct transfer of genetic material between bacteria is called conjugation. The host’s capability to conjugate vastly improves a plasmid’s chance of spreading in the bacterial population (Bennett, 2008).

Annually, hundreds of thousands of lives are being lost due to the declining strength of antibiotics (World Health Organization, 2012). A significant factor in the development of antimicrobial resistance is the overuse of antibiotics in medicine. Oftentimes antibiotics are prescribed to patients with symptoms stemming from viral infections – conditions against which antibiotic treatment is completely futile. It has been estimated that 60% of general antibiotic prescriptions are for the treatment of respiratory tract infections – a set of conditions that usually arise from viral infections (Lindbaek, 2006). In developing countries, antibiotics are usually obtainable without prescription (Hart and Kariuki, 1998). Loose medicinal legislation in poor and overpopulated areas may be imperative for people with no possibility to consult a doctor. The resulting misuse of antibiotics in turn favors the emergence of resistance. For example, the use of the antibiotic ciprofloxacin in developing countries has been showing alarming rise in resistant strains since the 1990s (Green and Tillotson, 1997; Rahman et al., 2014).

In addition to consumption by humans, the farm industry employs antibiotics in the treatment of sick farm animals. In many countries antibiotics are also pre-emptively administered by integrating them in animals’ diets – a practice already banned in the European Union (Clark et al., 2012). Not only does careless agricultural use drive bacterial

evolution in the animals, it also releases large amounts of antimicrobials to the environment.

3.1.2 Phage therapy

Bacteriophages are bacteria-infecting viruses. Even though phage therapy (PT) has been the subject of increasing speculation during the last two decades or so, the treatment method itself dates back to pre-antibiotic times. The notion of using phages in battle against bacteria was hypothesized as soon as phages were first discovered (d' Hérelle, 1926) and put to practice a few years later (Eaton, 1934). This novel form of therapy drew interest from industry and research worldwide until the 1930s and 1940s. At this time, antibiotics were discovered. Their ease of use contrasted with difficulties involved in PT studies; soon enough antibiotics were embraced as the evident remedy for curing infections. Consequently, interest on PT declined. Currently, it subsists as an approved treatment method only in Russia and Georgia and as an 'experimental treatment' in Poland (Levin and Bull, 2004; Pirnay et al., 2010). Phages are also used in the food-industry in the United States as preservatives (Sillankorva et al., 2012). The modern interest in PT began when the efficacy of antibiotics became questioned due to the alarming rise in nosocomial, antibiotic resistant bacterial strains. Pharmaceutical companies have nevertheless shown little interest in developing the treatment, as monetary requirements are high with moderately minor benefits to be expected (Thiel, 2004).

Recently, Jalasvuori and colleagues studied the effects of confronting antibiotic resistant bacteria with plasmid-dependent phages (Jalasvuori et al., 2011). This specific class of phages only infects plasmid-bearing cells. Unlike conventional phages, plasmid-dependent phages are capable of infecting a wide range of bacterial species, as long as the bacteria exhibit a plasmid-borne conjugation apparatus. Since plasmids often associate with antibiotic-resistance, this approach might prove useful in direct eradication of resistant cells. The study found that phage-dependent cells are highly effective in eradicating antibiotic resistance from a bacterial population. Some cells were also observed to lose the plasmid and consequently become resistant to phages and susceptible to antibiotics. Additionally, a small fraction of cells gained resistance towards phages while still retaining antibiotic resistance. Phage-resistance arose through the mutation of the conjugation apparatus, thus rendering the cell incapable of conjugation. The emergence of these mutants complicates population dynamics and gives rise to further questions. For

example, does the small population of phage-resistant bacteria still pose a threat to the patient? Is removing the ability to conjugate enough to tip the balance and eradicate the pathogen?

In light of the arising threat of antibiotic-resistance and promising recent discoveries in phage therapy, clinical trials following modern standards have become topical. Before such trials are initiated, *in silico* approaches may be used to review the role of phage therapy and other pre-emptive measures. The confined safety of computer circuitry allows us to test scenarios without having to account for the limitations of the real world.

3.2 Individual-based models

Traditionally, modelers have sought to formulate natural phenomena by strict mathematical means. The interpretation of mathematical models is relatively straightforward and they may provide elegant and general solutions for problems simple enough. For example, given that we know the present state of the planets in our solar system, we can accurately predict the motions of these heavenly bodies centuries ahead using simple and deterministic Newtonian dynamics (Barnes and Chu, 2010). However, if one wishes to truly capture the complexity inherent in the natural world, mathematical models become cumbersome. An ant colony provides a good example. In the colony, the population dynamics emerge from the complex interactions between thousands of social creatures and their environment. Each ant is an individual making seemingly independent decisions, yet the system, as a whole, is dependent on a dazzling number of interactions between these singular entities.

Complex systems, such as the ant colony, can be simplified using population-wide differential equations, but important details may become lost (Barnes and Chu, 2010). In theory, there is no limit to what differential equations may describe, but as the complexity of the system increases, the complexity of these equations climb exponentially (Bonabeau, 2002).

The age of efficient and inexpensive computing power has brought about a new approach to modeling complex systems. Although still in the minority, the proportion of individual-based models (IBMs) in modeling is on the rise (van Kleef et al., 2013). IBMs follow a bottom-up approach, where each individual comprising the heterogeneous population is considered an autonomous entity with distinct behavior. This is in contrast to mathematical population models, which compress the whole population to aggregate

equations, ignoring individual behavior. The simultaneous behavior and interaction of several entities give rise to the population and its emergent properties.

Emergent properties are fundamentally present in the natural world, yet not often emphasized by mathematical models (Bonabeau, 2002). Emergence arises as the result of the collective behavior of the entities. For example, the process of evolution may be considered an emergent property of a population. Imagine a virtual population of replicating entities, which are able to pass their attributes (heritability) with minor variations (mutation) to the next generation. An entity may have an attribute of speed, which affects its success in gathering resources. Those who lack speed may be wiped out due to hunger before being able to replicate (selection). Over time, one would expect to see increase in the mean speed attribute of the population. Yet nowhere is the increase in mean speed over generations scripted in the code. It is the collective result of heritability, selection and mutation that the evolutionary trend emerges.

Another key aspect of IBMs is the presence of stochasticity. Randomness is inherently present in the natural world, as revealed by the field of quantum mechanics (Pironio et al., 2011). Although randomness on a larger scale cannot be strictly inferred from quantum behavior, complex events on the macro-scale are often chaotic enough to be considered random (for example, throwing a dice). Randomness may also be present in mathematical models. However, in mathematical models the stochastic factor is applied to equations describing an aggregate of individuals, whereas in IBMs the variant may be precisely positioned to its righteous location: the individual (Bonabeau, 2002). For example, the decision-making process of an entity may involve complex calculations based on incoming data from different sensors (such as vision, hearing or touch). This data may stem from the randomized whereabouts of the individual or maybe an encounter brought about by the random movement of another individual.

The “accuracy” of IBMs comes with drawbacks; it is significantly harder to extract data from these models than from conventional mathematical models. The results obtained from IBMs are often numerical solutions: the results only apply to a specified set of parameters. This is in contrast to mathematical models, which are sometimes able to produce generalized results (Bonten et al., 2001). In a sense, IBMs take the *in silico* approach closer to more traditional methods of investigating the natural world. The

methods used to extract and analyze data borne similarity to extracting data from the real world, since the desired information is often buried under mountains of unwanted noise.

Despite their reliance on computational methods, IBMs are always dependent on some form of mathematics. This is also the case in the present model, as a sub-layer of the simulation describes bacterial dynamics inside a single patient using deterministic logistic growth equations. The line between IBMs and mathematical models is nothing short of blurry. It has even been suggested that differential equations describing the dynamics of the units of a system are together forming an IBM (Bonabeau, 2002). It is also common practice to fit both types of models in a single study (eg. Chakra et al., 2014) or to create analytical mathematical model based on IBMs (eg. D'Agata et al., 2007).

3.3 Modeling infections

In order to know which questions are worth posing and which ones have already been answered, it is worth going through some of the previously published hospital models. A study from 2012 used a mathematical model to investigate how the usage of antibiotics applied on bacterial infections contributed to the rise of an antibiotic resistant strain in a hospital setting (Grima et al., 2012). Specifically, the study focused on the treatment of *Clostridium difficile* associated diseases and the consequent rise of vancomycin-resistant enterococci. The model was built upon a previous model (D'Agata et al., 2005). Grima and colleagues state that since the variability and uncertainty of the parameters is rather high, sensitivity analyses became the main subjects of interest. These analyses revealed that the rate of antibiotic use, the duration of stay, hand-washing compliance and initial infection probability were to be the most sensitive variables in controlling the spread of nosocomial infections.

A study from 2000 by Lipsitch *et al*. presents a mathematical model with two antibiotics (Lipsitch et al., 2000). Their model allows for resistance to develop against drug 1, but not drug 2. The model assumes that patients receive antibiotics even if they are not colonized with the bacteria of interest, since the drugs may be used to treat a wide range of other symptoms. The experiment resulted in the un-intuitive result, that if transmission rate of bacteria between patients were high enough to maintain the presence of resistant bacteria, reducing the transmission rate would only reduce the prevalence of resistant bacteria and have no effect on sensitive bacteria. This is because resistant bacteria depend solely on transmission, whereas sensitive bacteria also gain population flow from incoming

patients. They also discovered that when a patient is treated with drug 2, they become likely to carry bacteria resistant to drug 1. This is surprising, because the level of resistance to drug 1 should be correlated with the usage of drug 1 and reversely correlated with drug 2. This is, however, reflected differently on the population level, as prevalence of resistance is reduced in the hospital as a whole due to usage of drug 2. This is a brilliant example of how population-level dynamics may possess unforeseen qualities. The study also concluded that clearing resistance from the hospital (weeks to months) is much faster than observed community-based infection interventions (several years).

Agata *et al*. studied the effects of minimizing treatment duration of patients. The IBM behind this study bears most similarity to the model presented in this thesis. To generalize the results of the IBM, a deterministic mathematical model was accompanied alongside the simulation. The authors concluded that minimizing the average duration a patient spends in a hospital has dramatic effects and may serve to fully eliminate the resistant pathogen from the hospital (D'Agata et al., 2007).

Hotchkiss and colleagues produced a spatial individual-based model of an intensive care unit (Hotchkiss et al., 2005). Their model relies on spatial order – that is, patients reside in spatially explicit rooms between which health-care workers move. Most infection models assume mass-action principles, which assume all agents to reside in a homogenous environment and their interactions to be based purely on fixed probability values. The model was merely created to present an example of how IBMs may be used to produce relevant data in optimizing health-care policies – the study therefore contains no quantitative or qualitative results.

4 Aims of the Study

The aim of this thesis was to create a virtual hospital simulation software and utilize it to investigate nosocomial infection patterns under various treatment and disinfection practices. Emphasis was in extending *in vitro* studies of plasmid-dependent PT to the virtual patient population level.

4.1 Specific questions

1. Can the conclusions drawn from the model be comfortably compared with existing studies?
2. What parameters are most sensitive in controlling nosocomial infections?
3. To what extent may pre-emptive disinfection protocols prevent the spread of nosocomial infections?
4. Should antibiotics and PT be used simultaneously or in succession?

5 Materials and Methods

5.1 The model in detail

Although mathematical models describing the spread of hospital acquired infections (see for example Ong et al., 2008; Haber et al., 2010; Austin et al., 1999b; Lipsitch et al., 2000; Webb et al., 2005 and review articles van Kleef et al., 2013; Magal and Ruan, 2014) are numerous, none of them encompasses the population and pathogen dynamics necessary for the purpose of this study. Thus, the model was built from scratch. The software was written in Java programming language (Sun Oracle microsystems) using Eclipse integrated development environment. The open source library opencsv was used to export the data into a spreadsheet-compatible format. Developing the model from the start made it possible to have full control of all of the underlying assumptions in the simulation. Main emphasis of the study was to construct a functional modeling software capable of probing a wide range of experimental settings. The actual studies performed with the simulator explore a number of interesting interactions, but more thorough investigations are left to future studies.

The simulation is based on interactions between patients, health care workers (HCWs), bacterial pathogens and antibacterial medicine (Figure 1).

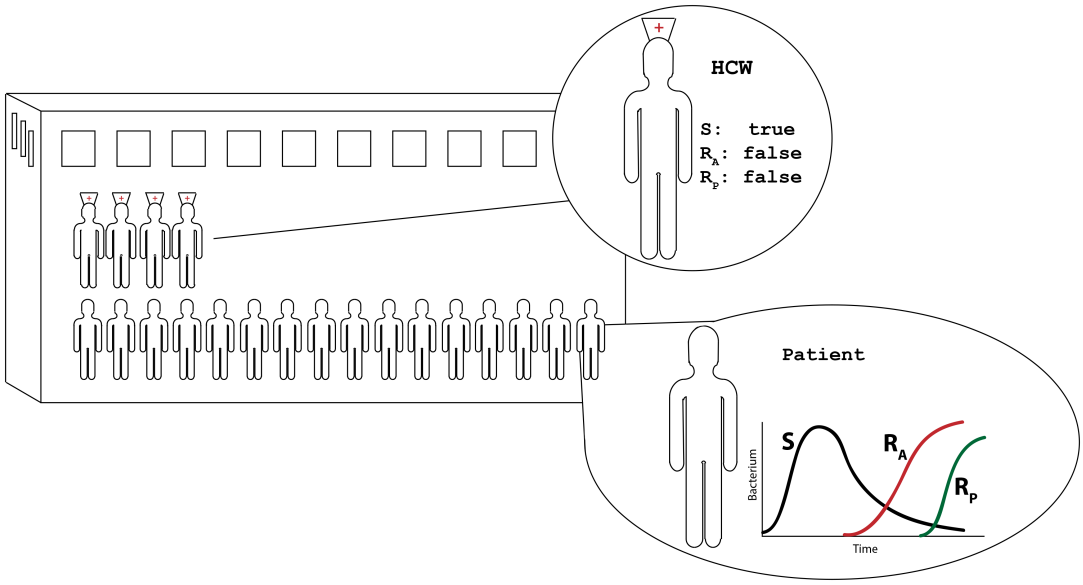

Figure 1. The hospital setting. Pathogens in patients are dynamically modeled, whereas health-care workers (HCW) are either carriers (true) or non-carriers (false) of a given strain. S, R_A and R_P refer to susceptible, antibiotic resistant and phage resistant bacteria, respectively.

To account for horizontal gene transfer and the emergence of resistant strains, the model includes several classes of pathogens. Due to the stochastic nature of events and the complexity involved in the interactions between bacteria, phages and their respective vectors, a purely mathematical approach was discarded in favor of a stochastic, individual-based model. The infection dynamics within a patient, however, are modeled using a mathematical approach. Most parameters described in the upcoming chapters are modifiable in the graphical user interface of the software (figure 2).

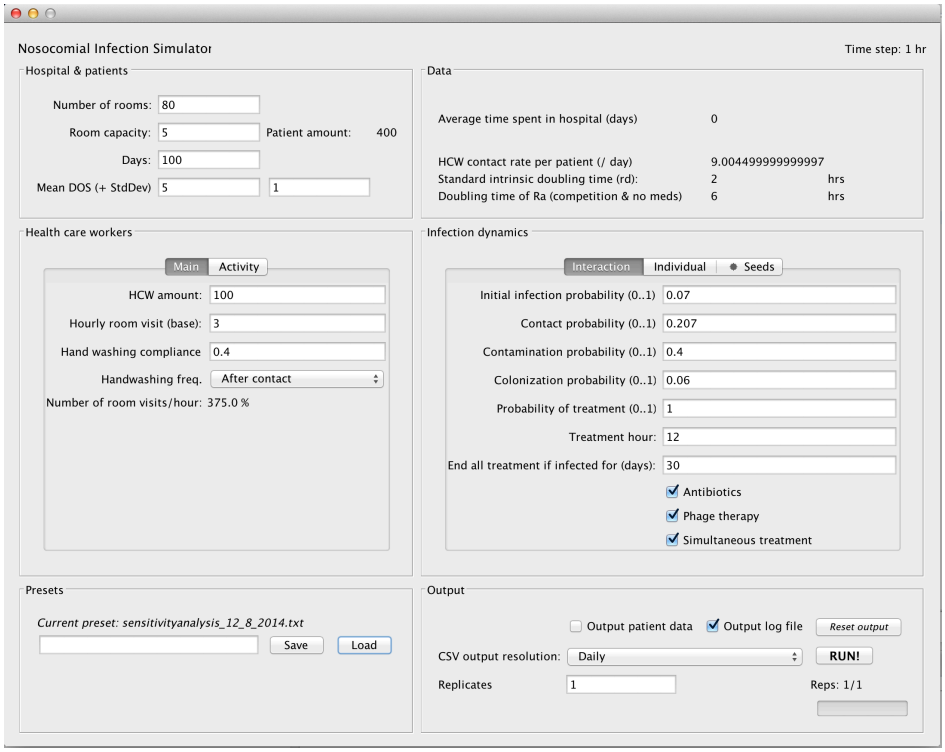

Figure 2. The graphical user interface of the simulator.

5.1.1 Patients

Upon admission to the hospital, each patient is assigned to a room and designated a duration of stay (DOS). The DOS determines the preliminary amount of days the patient is set to spend in the hospital, given that no infections occur. The average DOS across all patients is assumed to follow a normal distribution. The probability of an incoming patient being infected with susceptible bacteria is also determined (new patients are assumed not to carry resistant strains) – the overall percentage of initially infected patients consequently approaches this number. Each patient may theoretically house all three types of pathogens and be subject to antibiotics or phage therapy (PT) (figure 3).

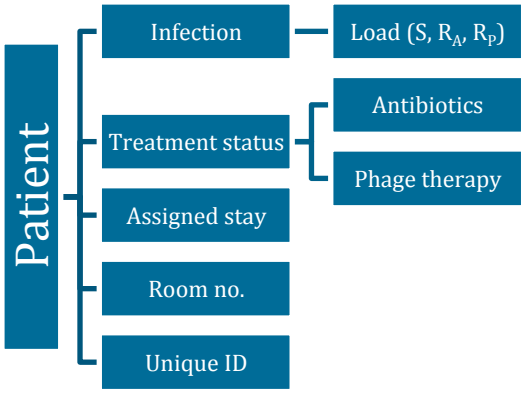

Figure 3. The attributes of a patient. Each patient may house all three kinds of pathogens. The patients may also be subject to antibiotics and/or phage therapy. Assigned duration of stay and room are determined when entering the hospital. The unique ID provides detailed logging of the actions of each patient

5.1.2 Health-care workers

Alongside patients, health-care workers (HCW) populate the hospital. In real life these may include nurses, physicians or any other people making day-to-day contact with patients. Although pathogens may be airborne and spread through ventilation, studies on the relative contributions of airborne and direct physical transmission show that the effect of the former is almost negligible in hospitals (Bauer et al., 1990). The model therefore assumes HCWs to act as the sole vectors of pathogen transmission. HCWs are never considered infected, since nosocomial bacteria usually target patients with wounds or weak immunity (Vincent, 2003). Consequently, the workers are referred to as ‘carriers’.

The efficiency of HCWs as vectors depends largely on disinfection practices, namely hand-washing compliance. Hand-washing compliance has been extensively studied and is indeed considered one of the most important practices in preventing the spread of nosocomial infections (Bauer et al., 1990). The software allows adjusting the compliance of a single HCW as well as a universal hand-washing frequency parameter, to which ‘compliance’ is measured against. Hand-washing frequency can be set to ‘after contact’, ‘after leaving a room’, ‘once an hour’, ‘once a day’ or ‘never’. When a hand-washing event is triggered, its success is determined by the compliance probability.

The movement of HCWs are built upon on a mass-action assumption: the spatial attributes of room-HCW interactions are random and arbitrary. This means that the room an HCW chooses to enter is picked randomly and is not affected by the previous space occupied by the HCW or by any other factor. However, the rooms may become incubational hot spots, since patients in a single room are more susceptible to an infection

arising in that room. This may favor the birth of sudden epidemics, stemming from a highly infected room. It is also possible to introduce heterogeneity in the activity of the HCWs: night shifts are obviously less crowded than day shifts. The software allows for hour-by-hour adjustment of the activity factor (see chapter 5.1.7).

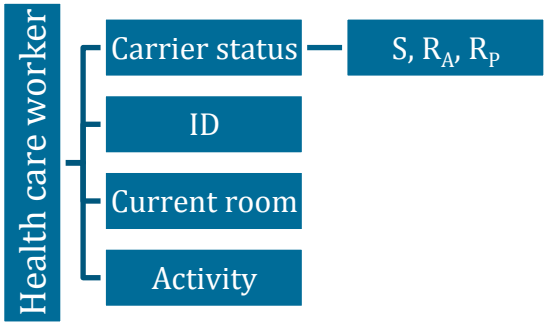

Figure 4. The attributes of a health care worker. Each may act as a carrier of all three types of bacteria. All carrier statuses are reset when hand washing occurs. At any given time, the HCW is located in a specific room. The activity of an HCW determines the number of room visits in an hour. Activity is a global parameter, which acts as stochastic factor in determining room visit probabilities for each HCW.

5.1.3 Pathogen types and mutation

The model defines three types of bacteria: Susceptible (S), antibiotic resistant (R_A) and PT resistant (R_P). Mutations allow each type to transform in various directions according to Figure 5. Mutation events will be discussed in the following subchapter.

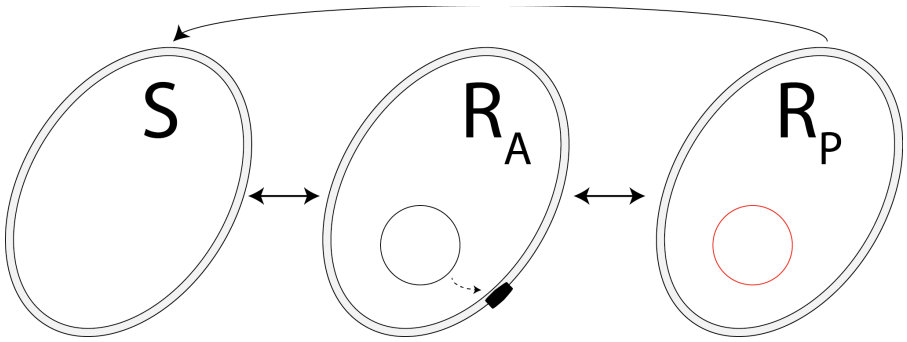

Figure 5. Bacterium types and mutation mechanisms. S = sensitive bacterium, R_A = antibiotic resistant bacterium, R_P = phage therapy resistant bacterium. The plasmid in R_A codes for a receptor utilized by bacteriophages (visualized on the cell wall). In R_P, the receptor is lost but the plasmid remains.

5.1.4 Within-patient pathogen model

A fraction of incoming patients are infected with sensitive bacteria (S). Once a bacterium is seeded upon a patient, logistic growth is assumed to take place. Logistic growth is a description of simple density dependent population dynamics (Tsoularis and Wallace, 2002). Growth begins slowly, but soon the binary fission of bacteria leads to exponential

growth. This continues as long as resources are plenty. Eventually the rate declines and stalls, as the carrying capacity of the environment becomes the limiter, resulting is an S-shaped curve. In a discrete time step model, this can be described with equation 1.

$$n(t+1) = n_t + rd \times n_t \left(1 - \frac{n_t}{K}\right) \qquad (1)$$

where n_t = bacterium population size at time t, rd = intrinsic growth rate and K = carrying capacity. The intrinsic growth rate (rd) determines the direction and velocity of growth. Rd is transformed upon the introduction of a competitive bacterial strain or due to the introduction of antimicrobials to a patient (Table 1 & 2).

A patient is subjected to antibiotic treatment when the patient's bacterium concentration marks a given value, termed the treatment threshold. The probability of the doctor successfully describing the medicine may be adjusted, allowing for realistic heterogeneity in medicine prescription timing. Once antibiotic treatment begins, a predefined value is subtracted from the rd of the bacterium (table 1). If this causes rd to go negative, the bacterium runs through its logistic growth curve in reverse, eventually disappearing altogether. The same principles apply to PT. If the R_A strain crosses the treatment threshold, PT is initiated and a pre-defined value is subtracted from the R_A's rd.

Table 1. Effects of medicine on different strains. The two treatment methods are shown on the columns and the three different strains on the lines. AbAdd and PtAdd are parameters describing the effectiveness of each treatment. For example, the effect of antibiotics on the S strain is that the growth rate (rd) of the bacterium is reduced by the predefined value of antibiotic effectiveness (AbAdd). The effectiveness values are negative, which is why summing is used instead of subtraction.

	Antibiotics	Phage therapy
S	Rd + AbAdd	-
R_A	-	Rd + PtAdd
R_P	-	-

Rd = intrinsic growth factor, S = susceptible bacterium, R_A = antibiotic resistant bacterium, R_P = phage therapy resistant bacterium, AbAdd = effectiveness of antibiotics, PtAdd = effectiveness of phage therapy.

When a patient is on antibiotics or PT, selection favors bacteria that withhold resistance towards the medicine. All mutations are equally likely all the time – if the mutation happens to take place during antibiotic treatment, emergent selection will favor the

resistant mutant. The probability of mutation is correlated with the size of the bacterial population, the mutation parameter referring to the probability of mutation when the bacterial load is at its maximum. For example, if S concentration is 20% of its maximum value, then the probability of S mutating to R_A (per hour) is 0.20 *times* [S to R_A mutation probability].

There are multiple ways a patient may be infected with resistant bacteria in the model. First, it is worth reminding that this model ultimately studies the effects of plasmid-borne resistance – that is, the resistance emanating under antibiotic treatment is assumed to rise due to the presence of a plasmid. Unlike some chromosome-dependent resistance mechanisms, the plasmid cannot be spontaneously created inside the bacterium. Instead, it is assumed to have originated from another strain or from the environment via horizontal gene transfer. The source is not specified further, but could be due to hospital visitors, the rare occurrence of airborne dispersal or infected patients coming from other hospitals. This model neglects the origin of the "first" plasmid for the sake of retaining the system in bounds of reasonable complexity.

In addition to the "spontaneous" introduction of a plasmid to an S-infected patient, the resistant strain may also spontaneously appear in healthy patients. The odds for this are assumed very low (defined by the parameter "R_A ground probability" in table 3). Having a potential plasmid host already present in a patient raises the probability of fixating a plasmid to a patient – therefore patients under S infection develop R_A bacteria more often.

However, the most important factor for the emergence of resistant bacteria is not spontaneous infection (although this is necessary to start the epidemic): the spread of bacteria from other patients act as the most important force spreading in resistant infections. As stated before, bacteria are spread between the patients by HCWs. Also, horizontal gene transfer (HGT) between co-existing bacterial types drives the spread of plasmids.

The simulator also allows for a different approach in introducing resistant pathogens. Instead of having constant mutation frequencies from S bacteria to R_A and from R_A to R_P, the timing of these mutations can be set to a fixed date. This is useful if patient and pathogen equilibrium is desired before introducing a new strain to the population. Also, rare events such as the appearance of an R_P bacterium through a mutation might have significant timing differences between replicates (R_P might first appear on day 100 and in

the second replicate on day 500). If these replicates are averaged, the resulting plots lose their characteristic shapes. This fixed introduction of pathogens is termed "seeding" and can be turned on and off by the user. Enabling seeding disables the corresponding mutation frequencies (S to R_A and R_A to R_P). Back mutations still remain effective.

When an antibiotic-resistant strain emerges inside a patient, it has the possibility to immediately affect the rd of the susceptible bacteria due to competition. The swap-time (time it takes for R_A to completely replace S) can be further decreased due to HGT. The transfer of the plasmid from R_A to S may be modeled by increasing the growth rate of R_A and consequently decreasing that of S. The extent of competition and HGT is definable by the user. Antibiotics do not affect resistant bacteria's rd and thus the antibiotic-resistant bacteria simply plateau on the carrying capacity, unless PT is employed. If R_A and S bacteria are present at the same time under no medicinal control, S may outcompete or hinder R_A. All pathogen-pathogen interactions are shown in Table 2, where effectors are listed in columns and effected strains on rows. For example, the effect of R_A bacteria on R_P is "Rd – UniComp", meaning that the rd of R_P bacteria is reduced by the universal competition constant (see Table 3).

Table 2. Pathogen-interaction table. Columns represent effectors and the rows the effected strains. For example, in the presence of S, the R_A strain has a reduced growth rate (rd) due to higher fitness of S. However, R_A is able to transform S to R_A by conjugation. If antibiotics are present, the rd of S is reduced.

	S	R_A	R_P
S	-	Rd - conjugation	-
R_A	Rd + conjugation Rd - SComp	-	-
R_P	Rd - SComp	Rd - UniComp	-

Rd = intrinsic growth factor, S = susceptible bacterium, R_A = antibiotic resistant bacterium, R_P = phage therapy resistant bacterium, SComp = competition factor of S, UniComp = universal competition factor (here referring to R_A's competition factor).

Fitness ranking obeys a rule where basic strains have negative impacts on the strains more "developed" on the fitness ladder. The extent or presence of fitness-heterogeneity is adjustable. The model allows adjusting the fitness of S relative to R_A and R_P (SComp), as well as R_A to R_P (UniComp) (for standard values, see table 3). The latter is termed as the

universal competition constant to reflect strain-types that might be implemented to the model in the future.

When a patient is infected with R_A, antibiotics are of no use. At this point the patient may be put on PT. As previously discussed, phages only target the plasmid-bearing resistant bacteria, R_A. The model does not distinguish the immediate action between antibiotics and PT in no other way than in their pathogen-fighting parameter and order of usage. The nomenclature of the two types of treatments could be reduced to drug 1 and drug 2, as in (Lipsitch et al., 2000). Accurately modeling phage therapy is complicated, since phages, similar to bacteria, are replicating entities with complex density dependent population dynamics (Payne, 2000). Combining the interactions of these two systems is beyond the scope of this study; the model presented here thus greatly simplifies the behavior of phages. What makes the current setting interesting, however, is the mutation dynamics between different bacterial types as shown in Figure 5. R_A is able to revert back to S or R_A, which could prove to have interesting consequences, depending on the medicinal status of the patient. Patient mortality was not modeled, since death rate due to nosocomial infections was assumed to be insignificant in a single hospital. This is also the approach followed by all the previously mentioned models.

5.1.5 Modeling pathogen spread between patients

Pathogens, whether susceptible or resistant, are capable of spreading inside the hospital using HCWs as vectors. Each time an HCW comes in contact with a patient, a predetermined probability value defines whether the HCW becomes a carrier of the bacterium currently residing in the patient. The HCWs remain carriers until the next hand-washing event. During an HCW's status as a carrier, each patient coming to contact with him is at risk of becoming infected with whatever bacterium the HCW is carrying. If the patient is infected with a bacterium of the same type, no transaction is necessary. If a patient infected with S becomes infected with R_A from an HCW, the inter-bacterial competition rules define the outcome (see chapter 7.1.4).

5.1.6 The day cycle

The hospital runs under a 24-hour day cycle, each hour comprising a single time-step in the model. Figure 6 illustrates the structure of each day.

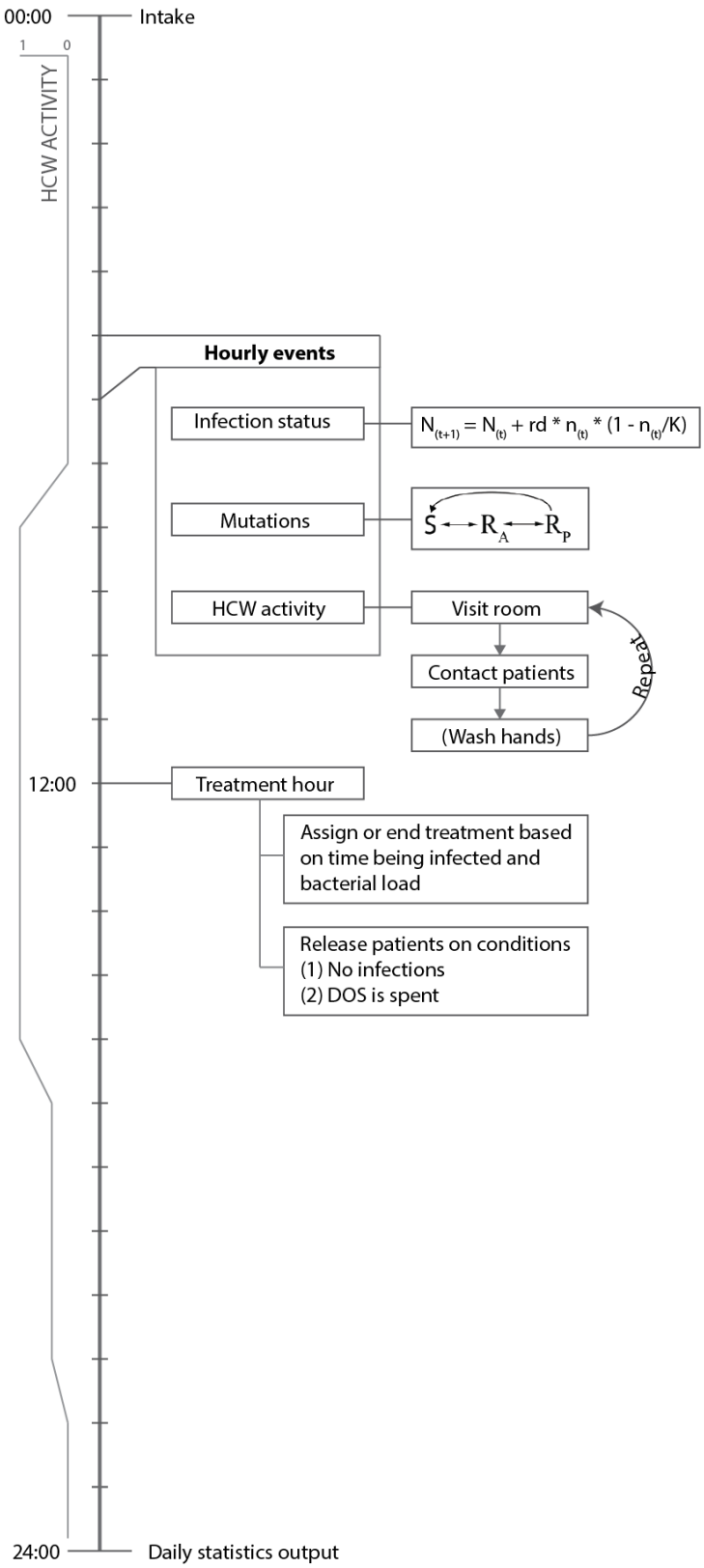

Figure 6. Daily cycle in the hospital. Hourly events take place 24 times a day (only one hourly event is shown for convenience). Time runs vertically, from top to bottom. HCW-activity is shown on the left (see also Figure 7).

‘Hourly events’ are performed once every time step. First, the bacterial load of each patient is recalculated. The medicinal and bacterial condition inside each patient are monitored and used to calculate the current growth rate of each residing bacterial type. Then, the previous bacterial loads and the freshly calculated growth rate are fed into equation 1, which outputs the current loads. All putative mutations take place after this. If a mutation does occur, the resulting mutant is seeded inside the patient by the amount defined by the inoculation parameter. This immediately affects the growth rate of other bacterial strains as defined by parameters in Table 2.

Once a day, each patient is subjected to a treatment event. This function applies actions on the patient based on their current infection status, time being infected and time spent in the hospital. The doctor can subscribe or terminate antibiotics or PT. The patient may also be released from the hospital, given that two conditions are met: (1) the patient must be clean of all infections and (2) their DOS must have been met.

Another special case of treatment is when a patient has been infected with any strain for an especially long period of time. This is usually the case, when a double resistant bacterium has taken over and saturated the patient. At this point, no medication is useful. The software keeps track of infection lengths and when a threshold-crossing infection period is detected, the patient is tagged “non-treated”: all treatment is stopped and the immune system of the patient is assumed to clear the patient of all pathogens. This is a fair assumption, given that the recovery period is long enough. If no immune system is considered, a single R_P bacterium would exponentially spread through the hospital and eventually saturate the whole patient population. Since no patient is released unless their infection status is zero, the hospital would come to a standstill with no in- or outflow of patients. Including the “non-treatment” tag imitates realistic practices, since most patients will eventually be cured or sent home – in rare cases do all patients spend the rest of their lives in the hospital due to a nosocomial infection. This may also reflect other pre-emptive measures, such as quarantine, which may be put forth in a real-life hospital.

As the hospital releases patients in a steady stream, an input is necessary to keep the population in equilibrium. At the beginning of each day, the output is met by exactly the same amount of input of patients, maintaining the population size at a constant. Standard time for patient input is set to 00:00.

5.1.7 Parameters

All the parameters in the current version of the model are listed in Table 3.

Table 3. Sources for the base values in parentheses: 1: (D'Agata et al., 2005) , 2: (Sørensen et al., 2001), 3: (Webb et al., 2005), 4: (D'Agata et al., 2007), 5: (Doebbeling et al., 1992).

Parameter	Function	Type/range	Base value
Days	How many days the simulation is run	Integer	-
Number of rooms	Number of rooms in the hospital	Integer	80 (1)
Room capacity	Number of patients in a single room	Integer	5 (1)
Mean DOS + StdDev	Mean duration of stay and standard deviation	Integer	5 (1) $\pm$ 1
Initial infection probability	The probability that an incoming patient is S infected	0…1	0.07 (4)
HCW amount	Amount of health care workers in the hospital	Integer	100 (1)
Hourly room visit (base)	Number of rooms visits/hr assuming activity of 1.0	>= 0	3
Hand washing compliance	Probability that an HCW washes hands	0…1	0.4 (5)
Hand washing frequency	The frequency of hand washing events	list	After contact
HCW activity (hourly)	Defines actual visits/hr by multiplying the base value	0…1	See Figure 7
Contact probability	Probability of HCW contacting a patient when in a room	0…1	0.207 (1)
Contamination probability	Probability of HCW contamination in contact	0…1	0.4 (1)
Colonization probability	Probability of patient infection in contact	0…1	0.06 (1)
Max load	Maximum bacterial load (K in logistic growth eq.)	Double	$1.1x10^{11}$ (3)
Infection threshold	Threshold for patient being infectious	Double	10^{11} (1,2)
Standard rd	Default intrinsic growth factor, when no effectors (competition or medicine) present	> 0	0.413
Antibiotic rd add	The effect of antibiotics on S bacteria's rd	< 0	-0.5 (3)
Phage therapy rd add	The effect of PT on R_A bacteria's rd	< 0	-0.5
Inoculant	Amount of bacteria upon infection	> 0	10^6 (2)
Non-treatment tag add	Universal rd when patient is tagged non-treated	< 0	-0.03
S comp. factor	Superiority of S against other bacteria, when antibiotics are not present (i.e tradeoff from resistance)	<= 0	-0.2905 (3,4)
Universal comp. factor	Superiority of resistant bacteria, when under selective pressure	>= 0	-0.2905 (3,4)
Conjugation constant	Change of growth rates due to HGT from R_A to S	>= 0	0.1
R_A ground probability	Probability of spontaneous emergence of R_A in healthy patient	>= 0	10^{-8}
S to R_A mutation prob.	Probability of S transforming to R_A	>= 0	10^{-3}
R_A to R_P mutation prob.	Probability of R_A transforming to R_P	>= 0	10^{-4}
R_A to S mutation prob.	Probability of R_A transforming back to S	>= 0	10^{-3}
R_P to S mutation prob.	Probability of R_P transforming back to S	>= 0	10^{-4}
Treatment threshold	The point of the infection cycle when medicine may be described	Double	10^{11}

Probability of treatment	The probability of the doctor prescribing medicine	0…1	1.0
Treatment hour	Hour of day when each patient sees "the doctor"	0 - 24	12
End all treatment if infected	Infection time threshold for tagging the patient 'non-treated'	days	30
Antibiotics	On/Off	Boolean	-
Phage therapy	On/Off	Boolean	-
Simultaneous treatment	Whether antibiotics and PT are used simultaneously	Boolean	-
R_A seed	The date for initial R_A infection. Overrides regular mutation mechanisms.	day	150
R_P seed	The date for initial R_P infection. Overrides regular mutation mechanisms.	day	200

The base values shown in Table 3 are the standard values used in the forthcoming experiments, unless otherwise stated. The values are derived from a handful of studies representing different hospital settings. The bacteria are assumed to be types of species, which live on the skin, respiratory tracts or digestive systems of humans as described in Lipsitch et al. (2000). The proportion of incoming patients already infected with S varies greatly from study to study. The value of 0.07 estimated by D'Agata et al. (2007) was eventually chosen as the base value. The infection threshold of the bacterial load was approximated to be 10^{11} as in D'Agata et al. (2007) and Webb et al. (2005). Inoculation amount is set to 10^6 cells (Sørensen et al., 2001; D'Agata et al., 2007).

Most models describing bacterial growth within a patient express growth rate as 'doubling time'. The current model primarily uses the growth rate parameter due to its compatibility with the logistic growth equation. For practical purposes, the software also calculates and displays the doubling time using the formula $\frac{\ln(2)}{\ln(1+rd)}$ (D'Agata et al., 2006). Rd is then approximated to be 0.413 for susceptible bacteria (before treatments) to provide a doubling time of two hours as shown by Webb et al. (2005).

The superiority of S bacteria against other bacteria under no medicine simulates the tradeoffs brought about by resistance (Table 2). This advantage in fitness remains even while under antibiotic treatment, but since antibiotics efficiently eradicate S bacteria, the less-fit strains are not affected for long – as soon as S bacteria disappear, the rd of R_A and R_P bounce back to their original value (unless medication or competition between R_A and R_P have effects). The baseline competition values (the S competition factor is set to -0.2905) provide a doubling time of approximately six hours for R_A/R_P bacteria under no

medication (D'Agata et al., 2007; Webb et al., 2005). More complex fitness-relationships may be explored in future versions of the model.

The conjugation cofactor (base value 0.1, estimate) simulates HGT between resistant and non-resistant bacteria. This transforms S bacteria to the R_A class, speeding up the growth of R_A and decaying the S population. The model approximates the maximum load $1.1 * 10^{11}$ (the logistic growth equation requires the maximum load to be larger than the infection threshold, hence the factor 1.1). Antibiotics reduce rd of S bacteria by 0.4, leading to an rd of -0.05. For PT the reduction is assumed similar. In practice, this causes a fully saturated patient to be cleared of the bacterium in approximately in ten days (Webb et al., 2005).

Resistant bacteria may emerge in a patient through spontaneous infection or through vector (HCW) mediated transfer. The former is defined by the parameter 'R_A ground probability' for patient with no infection and by 'S to R_A mutation prob.' for patient already colonized with S. Again, the mutational parameters refer to patients being fully saturated with the original strain. The mutation probabilities between different bacterial types are based on estimates. One of the goals of this study is to explore the parameter space for interesting combinations of mutational probabilities.

The treatment threshold currently equals the infection threshold, since this is the point when the doctors are assumed to notice infections and prescribe treatment. The timing of treatment hour is not arbitrary – it strongly affects global infection dynamics. The later the event is set, the more time the patient has to spread the pathogens via HCWs. This is aspect is discussed in detail later.

The amount of interaction between HCWs and patients is usually represented by contact rate per patient (per unit time). Previously, the rate was determined to be 8-10 contacts per patient in a day, depending on the infection status of the patient (D'Agata et al., 2005). Due to the object-oriented simulation approach implemented in this model, the contact rate cannot be explicitly inserted as a parameter. However, transformation between the two types of systems is straightforward. The contact rate per person (/day) can be determined with equation 2.

$$Contact\ rate = \sum_{t=1}^{24} Activity(t) \times \frac{room\ visits\ per\ hour}{number\ of\ rooms} \times HCW\ number \times contact\ prob \qquad (2)$$

The base values in Table 3 are calibrated so that the contact rate settles around 9 – the average value used by Agata et al. (2005). The software automatically calculates the rate and displays it in the data section of the graphical user interface, so implementation of parameters derived from previously published studies is easier.

HCWs are not assumed to be uniformly active throughout the day. Figure 7 shows a proposed model of HCW-activity. This model has no backing data and is simply an estimate. The activity factor multiplies the "hourly room visit" –parameter. This is then used to obtain the actual number of room visits per hour for each HCW. The activity table in Figure 7 is used as the base value for all forthcoming experiments.

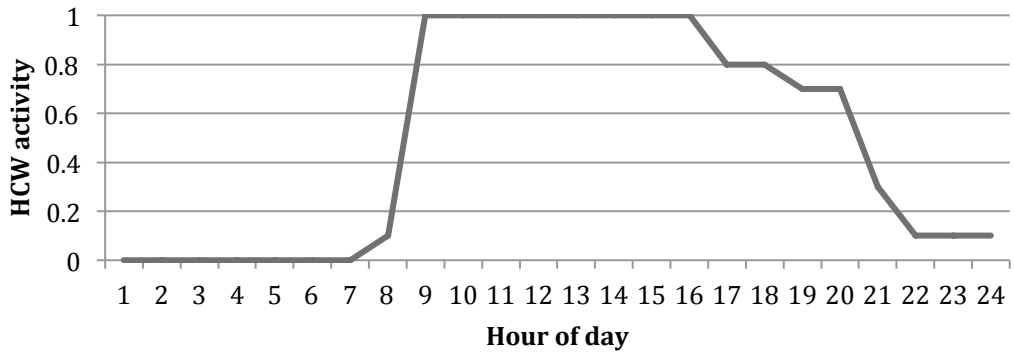

Figure 7. An approximation of HCW-activity through a single day. X-axis shows the hour of day. The activity on the Y-axis is the factor determining the number of room visits in an hour.

5.2 Study questions

5.2.1 Comparison with existing studies

The capability of the model to reproduce data from previous studies was investigated. The parameters were set to replicate an *in vivo* study by Austin et al. (1999b). The authors observed the prevalence of vancomycin resistant enterococci in a real-world intensive care unit during a period of 133 days. Standard values as shown in Table 3 were used, with the exception of hand-washing compliance (51%) and initial infection probability (15%), as reported by this particular study (Austin et al., 1999b). The simulation was repeated ten times and the proportion of infected patients was determined after 133 days. For simplicity, possibility of further resistance is not allowed: VRE is represented by the S strain, which can be killed using other antibiotics, such as ampicillin (Quale et al., 1996).

The within-patient model was tested against a previously published mathematical model (Webb et al., 2005). This model was used to fine-tune the current within-patient

model. Growth rates, competition factors and medicinal effectiveness were calibrated to meet their data. Since the within-patient model is mathematical and deterministic, no simulation replicates were necessary.

5.2.2 Sensitivity analysis

One-factor-at-a-time sensitivity approach (Saltelli et al., 2000) was used to asses the relative importance of multiple parameters to the overall pathogen prevalence. The results were studied under linear regression, where the average number of days spent in the hospital is plotted as a function of the varying parameters. Seven interesting parameters were chosen: initial infection probability, HCW compliance, HCW contact probability, contamination probability, colonization probability, efficiency of antibiotics and patient:HCW ratio. The base-values were scaled from 10% to 200% with intervals of 10 %. The treatment probability parameter was also studied. Since its base value is 1.0, this probability value could not be scaled too 200%. This parameter is therefore plotted separately.

This experimental setting makes the simplifying assumption that S bacteria are the only pathogen type. This is done to minimize any masking factors that may arise from having multiple pathogens and different types of medicine - the goal is to explore which parameters are most important in reducing nosocomial infections in general. The values left unchanged are listed in Table 3; the varied values are fractions or multiples of the base-values. Each setting was replicated four times for 100 days. Average values and standard deviations were calculated.

5.2.3 Effects of pre-emptive disinfection procedures

In addition to the sensitivity analysis above, the effect of hand washing compliance on the overall prevalence of S was investigated. The hand washing compliance parameter was varied from 0 to 1 with intervals of 0.1. Hand-washing frequency was set to ‘after contact’. Five replicates were done to quantify stochasticity. Prevalence of S was determined from the stable state and plotted as a function of hand-washing compliance.

5.2.4 Timing of treatment

The timing of the treatment event may have important consequences in pathogen prevalence and thus the influence of timing to the efficacy of the treatment was investigated. The timing of the treatment was scheduled to start at different stages in

separate simulations and the corresponding average duration of stays were recorded. Since variation was observed to be low between different runs, only two replicates were done for each time step (1 hour). All parameters values followed the base values in Table 3. For simplicity, this experiment also discarded other bacterial types than S.

5.2.5 Simultaneous or successive: the order of treatments

The software was used to review different strategies of patient care and discover how they reflect on the population level. Two treatment strategies were scripted in the model: antibiotics and phage therapy can either be used in succession or simultaneously. If a patient becomes infected with S while the system is running under successive strategy, he/she is treated with antibiotics and no PT. Detection of an antibiotic resistant strain then ensues the deployment of PT and immediately cuts off antibiotics. While under simultaneous treatment, both treatments can be administered regardless of each other. If R_P has been prevalent for more than 30 days, the patient receives the "non-treated tag". The simulations were run for 400 days using the base values in Table 3. Two sets of five replicates were done. The sets differed in the treatment strategy used.

6 Results

6.1 Comparison with existing studies

The results derived with the current model were compared to the results of a previous study by Austin et al. (1999b), who determined the overall prevalence of vancomycin resistant enterococci to be 36% after 133 days. Given the customized starting conditions, the current model estimates the S strain to find equilibrium around 32 % (± 2.6%).

The mathematical within-patient model created by Webb et al. (2005) served as the basis for calibrating the current within-patient model. When a patient is infected, the pathogens take approximately three days to reach the carrying capacity – a similar result was observed in the current model. Similarly, antibiotics effectively eradicate the pathogen in approximately ten days in both models.

6.2 Sensitivity analysis

The sensitivity of model parameters was studied by varying each parameter around their base-value. In Figure 8, the average number of days a patient spends in the hospital is plotted against seven different parameters, whose base-values are scaled from 10% to 200%. Figure 9 displays the effect of treatment probability on the actual average duration of stay.

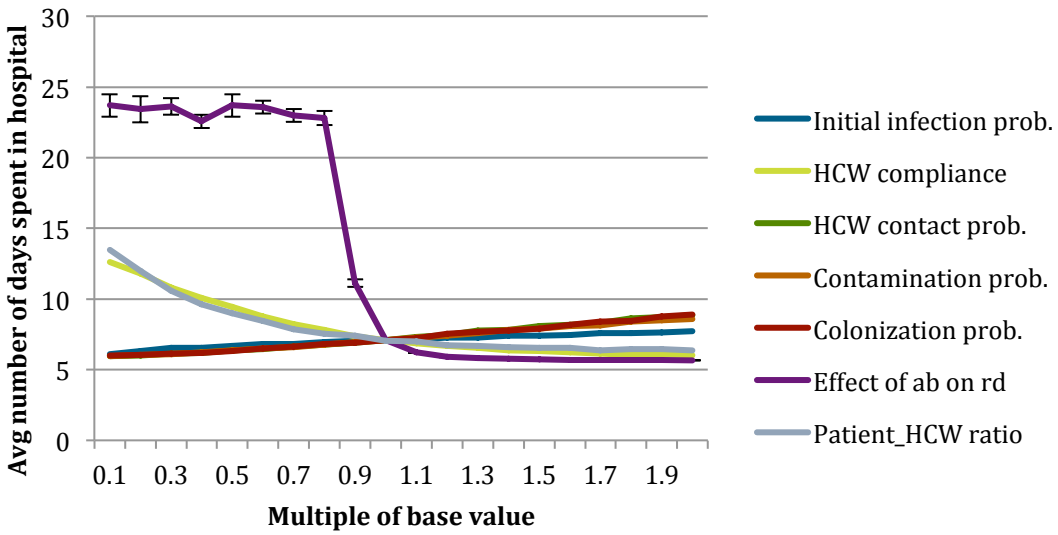

Figure 8. Sensitivity analysis of seven parameters. Each parameters was varied from 10 to 200% of its base value by steps of 10%. Only standard deviations exceeding 0.2 days are shown for convenience.

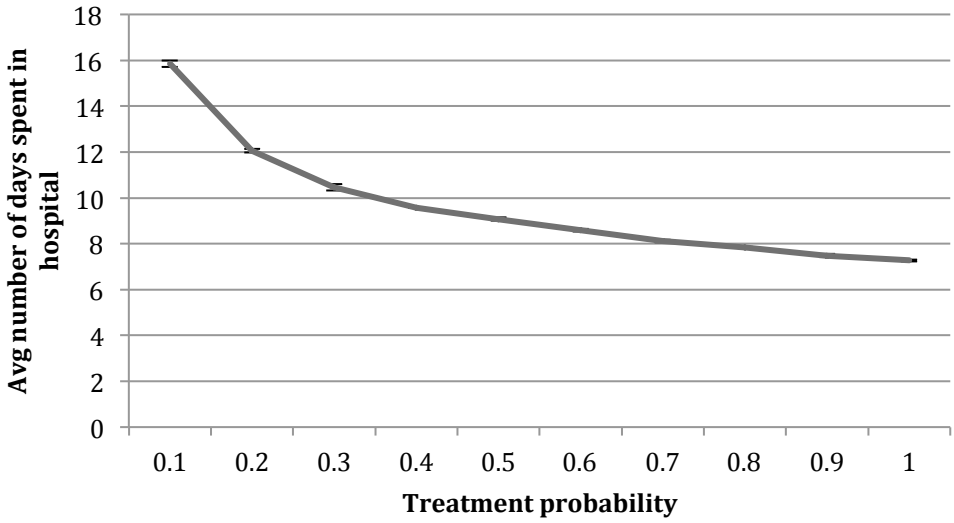

Figure 9. The logarithmic relationship between Treatment probability and days spent in the hospital. Standard deviations shown.

6.3 Effects of pre-emptive disinfection procedures

Disinfection procedures were studied further by investigating how hand-washing affects the overall prevalence of S. Compliancy was varied from zero to 100% and the overall prevalence of S at equilibrium was then plotted as a function of compliance (Figure 10).

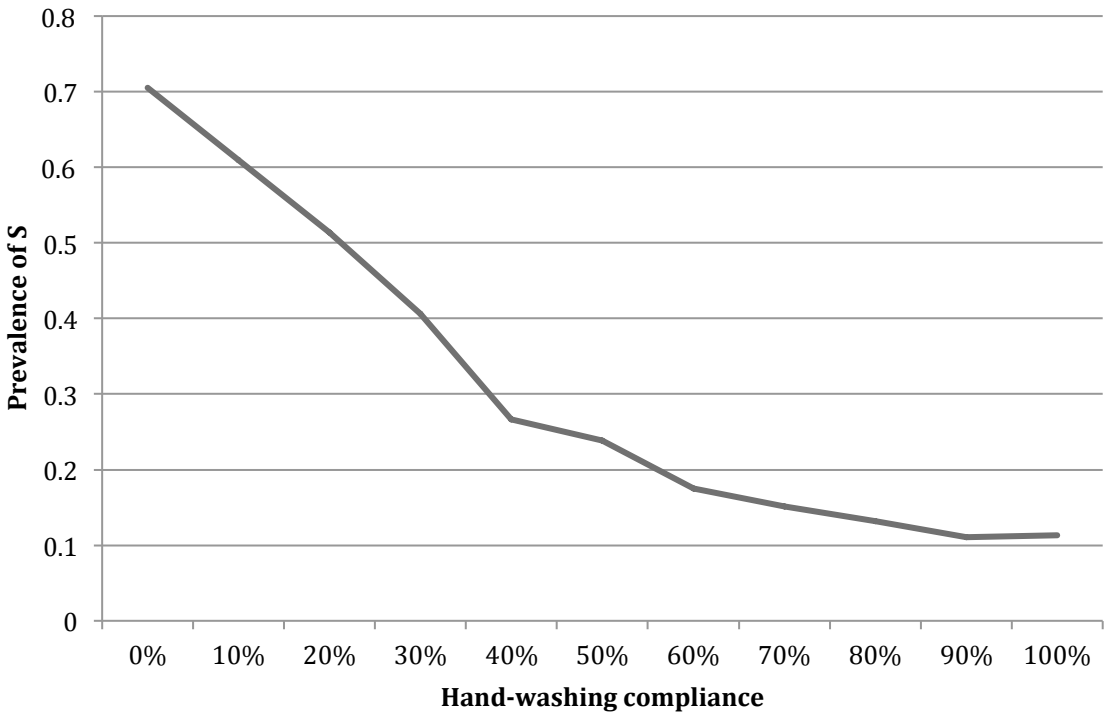

Figure 10. The effect of hand-washing compliance on the overall prevalence of S, as calculated from equilibrium. Compliancy refers to the probability of a health-care worker washing hands after contacting a patient. Standard deviations are not shown (less than 0.04 days).

6.4 Timing of treatment

Timing of treatment was varied to discover its effect on the average number of days a patient spends in the hospital. Varying the treatment event's timing results in an ascending curve (Figure 11). Two replicates per hour were done and averaged.

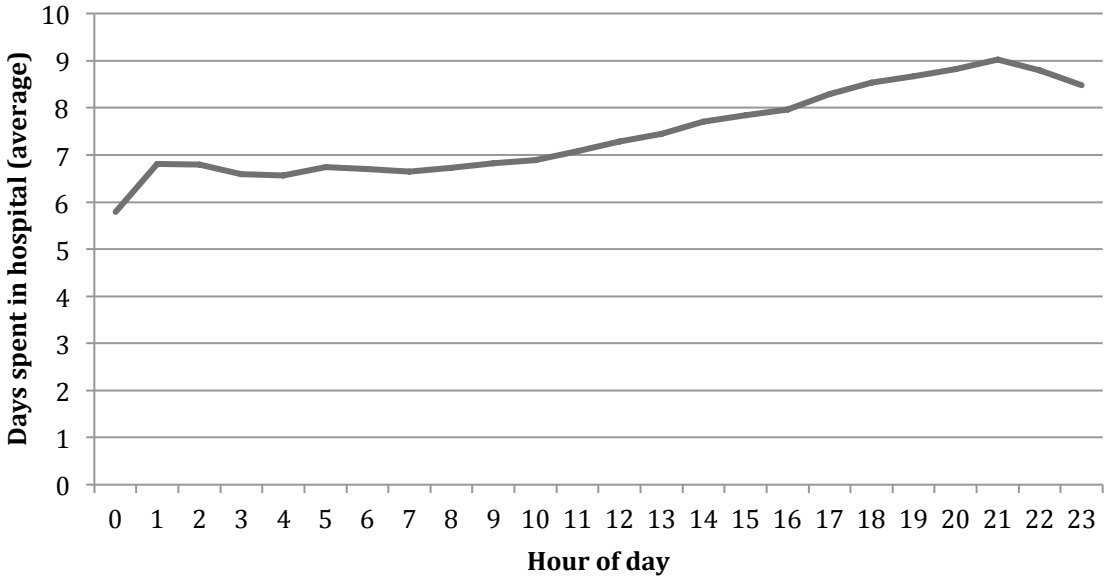

Figure 11. Average amount of time spent in hospital as a function timing of the treatment event. Standard deviations not shown, due to their minimal size (average 0.041 days).

6.5 Simultaneous or successive

Running the simulation using two different treatment strategies resulted in the values shown in table 4. Additionally, the average durations of stay were observed to be 7.95 (±0.06) days and 9.1 (±0.27) days in simultaneous and successive treatments, respectively. Simultaneous treatment reduces the average number of hospital days by approximately 12.5 %. The prevalence of S is lowered by 29 % and the prevalence of R_A by 10 %.

Table 4. Effects between simultaneous and successive prescription of antibiotics and PT. Percentages are means of five replicates (± STD).

	Prevalence of S	**Prevalence of R_A**
Simultaneous	26.8 % ±1.67 %	10.6 % ±1.6 %
Successive	37.9 % ±3.6%	11.9 % ±1.8%

S = susceptible bacterium, R_A = antibiotic resistant bacterium.

7 Discussion

7.1 Comparisons with existing models

Comparing the results produced by this model with data from previous studies is central to establishing an estimate for the accuracy of the model. Comparison with the study done by Austin et al. (1999b), indicate that the present model is capable of producing results of a similar scale as a real-world study. Using the calibrated base values predicts remarkably similar overall prevalence of S. It is worth noting that many of the base values have origins in other studies and may as such fail to accurately the describe the behavior of a specific hospital setting. Slight manipulation of the base values has severe consequences. For example, setting the treatment hour to 00:00 instead of 12:00 decreases the prevalence down to approximately 20%. Thus, whether the similarity in results between this simulation and the study by Austin et al. (1999b) is due to a lucky balance of parameters or whether it indicates great accuracy of the model is an issue to be solved by further calibrating the model to fit other real-world studies.

The within-patient model was calibrated using data from Webb et al. (2005). As the present within-patient model is by its nature a deterministic one, comparison with its deterministic peers is straightforward: the models, by definition, produce similar results given identical starting conditions. The positive results serve to reassure that the growth mechanisms and patterns are valid.

7.2 Sensitivity analysis

Sensitivity analysis is a commonly used technique in determining the proportional effects of parameters in a model. As shown by figure 8, the seven parameters have somewhat distinctive shapes and scales. The probability at which HCWs wash their hands (HCW compliance) has an exponential effect on the average number of days a patient spends in the hospital. Interestingly, a similar curve is observed in the Patient-HCW ratio parameter: the more there are HCWs, more efficient is the spread of disease. Theoretically, an HCW-free hospital would, then, serve as the optimum environment for preventing the spread of nosocomial infections. Obviously, this is not true and merely goes to show that in this particular model the positive effects of HCWs are not modeled.

Most other parameters exhibit linear relationships and are mostly overlapping (initial infection probability has a slightly less of an effect than the other linear parameters). The peculiar shape of “Effects on ab on rd” is due to the fact that as the value of this parameter crosses the rd value of an infectious bacterium, the growth of the bacterium becomes negative. When the value of “Ab rd add” is less than the rd value, the antibiotics are not sufficient to clear an infection and the patient are eventually released through the “non-treated” procedure.

The probability of treatment has a profound effect on the number of hospital days (Figure 9). Since the value of this parameter is assumed 1.0, the spectrum of sensitivity analysis values was only varied below 1.0 (a probability value cannot exceed 1.0). It is for the same reason this was plotted separately.

It is worth nothing that the parameters in Figure 8 are scaled with reference to their base value. If a broader spectrum of values were to be used, more profound effects would be observed. However, this sensitivity analysis experiment shows how realistic manipulation of the base values, which are assumed to approximate real-world values, may yield dramatic effects on the average number of hospital days. The separately plotted treatment probability parameter was studied under a larger scale of values (absolute values from zero to 100%) than other parameters and as such may not be fully comparable in a sensitivity analysis.

7.3 Implications of hand-washing on pathogen prevalence

Hand-washing compliance is a major factor in the spread of nosocomial infections, as shown by the sensitivity analysis and previous studies. In addition to studying the effects of compliance on the number of hospital days, it is also worth seeing how it affects the overall prevalence of pathogens. Modeling the implications involved doing a series of replicates on a range of compliance values. As figure 10 shows, the prevalence of S bacteria is reversely correlated with hand-washing compliance. Interestingly, having full compliance is not enough to fully eradicate the pathogen. The reason is the steady flux of incoming patients who provide a steady source of S bacteria. Similarly, having no hand washing at all doesn’t result in total saturation of the patient population with S. Instead, pathogen prevalence finds equilibrium at around 70%. This is due to the fact that patients remain infectious only for a fraction of their total infection time. After antibiotic treatment

has begun, the infectiousness quickly lowers and the health-care workers can no longer be contaminated.

7.4 Timing of treatment event

As the global treatment event is pushed further through the day, the average time spent in the hospital increases. The increase is most prevalent during daytime, since it is the active time of the HCWs (figure 7). If the model employed a uniform HCW-activity table, the increase in average time spent in hospital would most likely become linear. The most single dramatic rise in hospital days occurs during the first hour of day. This reveals the fact that early inhibition of pathogen dispersal is crucial.

The positive effect of early treatment may seem trivial, but still calls for more detailed examination of the model mechanisms giving rise to it. All incoming patients are spawned to the hospital in the beginning of each day – that is, at midnight. Since treatment hour is assumed to be sometime during the day, the incoming patients that are already infected with the S-strain are free to spread their pathogens throughout the night (assuming there is HCW activity during the night). If treatment hour is set earlier, say midnight, the incoming patients have no means of spreading the disease since antibiotics are very quick at lowering the bacterial loads below the level of infectiousness. In real life, all patients are obviously not treated simultaneously nor do all the patients arrive at midnight – the treatment hour represents another simplifying assumptions of the model. The assumption serves to caricaturize the effect of the timing of treatment, even if it fails to provide realistic numerical data. The onset of treatment has also been studied by Agata and colleagues, who came to the conclusion it may have important inhibitory implications on the spread of resistant pathogens (D'Agata et al., 2007).

7.5 Simultaneous or successive

Let us consider discontinuing antibiotics upon starting PT. In this scenario, no S bacteria are assumed to remain due to antibiotics and competition. Therefore, continuing the use of antibiotics would be a waste of resources. In addition, the prevalence of antibiotics at this point may accelerate resistance-development in other bacterial strains within the patient and also disturb commensal intestinal flora (Levin et al., 1997). Based on these assumptions, the non-simultaneous usage of antibiotics and PT would seem reasonable. However, the possibility of R_A reverting to S is present. In this case, the novel S strain

would thrive in an antibiotic-free environment. The initial coexistence of S and R_A can also not be ruled out. In successive treatment, no selective pressure would suppress the growth of S and the infection cycle could start all over again. This might lead to an oscillating cycle between the two bacterial types. For patients with weak immunity, additional infection cycles could have severe consequences.

If antibiotics are continued alongside PT (simultaneous mode), the selective pressures are directed so that the only plausible additional phenotype would be a bacterium resistant both to antibiotics and to PT: R_P (Jalasvuori et al., 2011). Such a strain could, of course, also emerge under successive treatment. An important point to notice, however, is that if R_P reverts to S under successive-treatment mode, the S strain might outcompete R_P in the resulting antibiotic-free environment. In simultaneous mode, S is always suppressed and will never subdue R_P by competition.

A previous study done on the simultaneous use of phages and antibiotics shows significant improvement in overall efficiency of the treatment (Zhang and Buckling, 2012). Another study shows that the order in which the two treatments are administered is important (Escobar-Páramo et al., 2012). As previously mentioned, the effect of plasmid-dependent phages on the prevalence of antibiotic resistant microbes is notable *in vitro* (Jalasvuori et al., 2011). The current model verifies these findings, since the prevalence of pathogens at equilibrium is lower and average duration of stay is shortened when using simultaneous treatment. Interestingly, the reduction in the prevalence of S-bacteria is almost threefold as compared to reduction in R_A. This unintuitive result stems from the mutational mechanisms (figure 5) and the fact that the model prioritizes phage therapy over antibiotics. It is more common for patients to be infected with S bacteria prior to being infected with R_A. When a patient then becomes infected with R_A, antibiotics are discontinued and the S strain once again saturates the patient. The infection times for S are longer, because R_A must first be fully eradicated before the patient is again administered antibiotics.

Whether this result is completely due to the built-in assumptions of the model or an actual reflection of real-life phenomena serves basis for speculation. The complete exclusion of antibiotic treatment while under PT is no doubt an artificial setting - the overlapping of the two treatments most likely follow a spectrum instead of confining to strict binary extremes. However, in cases of a double infection, prioritizing the treatment

of a more pathogenic strain of bacteria is a fair assumption given that the treatments do not occur simultaneously. Such a treatment plan may arise simply from ignorance of the existence of another strain. The secondary strain may also appear after the treatment plan has been made, due to mutations, HGT or vector-mediated transfer. It should also be considered that employing antibiotics alongside PT for the sake of reassurance contradicts the policy of reducing global antibiotic use. Simultaneous use should therefore be always justified. The results from this particular test are missing p-values, since statistical significance tests are not considered compatible with stochastic computer simulations (White et al., 2013).

7.6 Conclusions

The simulator reveals several important factors in maintaining an infection-free hospital. Early diagnosis of infections plays a major role in minimizing the prevalence of bacterial pathogens. Compliance of HCWs in disinfection-practices is crucial, but does not alone determine the prevalence level of pathogens. Combining antibiotics and plasmid-dependent PT decreases the overall prevalence of susceptible and resistant pathogens, with emphasis on the former.

These results illustrate the types of scenarios the simulator may be used to replicate. Substantiation of the results would require further fine-tuning of the parameters and preferably co-operation with an actual hospital. In its current form the software provides a functional platform for a wide range of infection simulations ranging from the individual to the population level. The model has been highly customized for the specific type of infections and dynamics that were the initial catalyst for creating the software – namely phage therapy.

At the cost of specification, the model lacks generality. Major future improvements could include the possibility of adding unlimited types of pathogens, vectors and hosts. All aspects of dispersal, mutation, competition and medicine could then be freely adjusted. A future model might reduce to something that is not specifically tailored for hospital infection modeling, but a general engine for simulating vector-borne infections. More advanced spatial attributes could also be added along with visual representations of spatiality. This would allow for predicting how pathogens move within hospitals and what quarantine procedures prove effective. The next generation of the model could be designed

with emphasis on generality, extendibility and visual output. A command line interface could also be implemented.

As power in computing continues to grow, models will be able comprise increasingly more complex interactions and thus, in principle, yield more accurate predictions of our world. However, as complexity increases, uncovering realistic parameter values must be given paramount consideration. Incorporating a new parameter always calls for judgment on whether its integration is indeed justifiable, given the complexity and increase in noise its addition may cause. However, when acknowledging the positive aspects of simplicity, individual-based models may prove to be extraordinarily useful in modeling complex systems. The simulator presented here has been a fusion of various modeling perspectives, but mostly driven by the individual-based modeling approach. In the software, the level of complexity is adjustable simply by making parameter values uniform or ineffective.

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