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Agent-based model of Fecal Microbial Transplant effect on Bile Acid Metabolism on suppressing *Clostridium difficile* infection: an example of agent-based modeling of intestinal bacterial infection

Xavier Peer and Gary An*

Abstract

Agent-based modeling is a computational modeling method that represents system-level behavior as arising from multiple interactions between the multiple components that make up a system. Biological systems are thus readily described using agent-based models (ABMs), as multi-cellular organisms can be viewed as populations of interacting cells, and microbial systems manifest as colonies of individual microbes. Intersections between these two domains underlie an increasing number of pathophysiological processes, and the intestinal tract represents one of the most significant locations for these inter-domain interactions, so much so that it can be considered an internal ecology of varying robustness and function. Intestinal infections represent significant disturbances of this internal ecology, and one of the most clinically relevant intestinal infections is *Clostridium difficile* infection (CDI). CDI is precipitated by the use of broad-spectrum antibiotics, involves the depletion of commensal microbiota, and alterations in bile acid composition in the intestinal lumen. We present an example ABM of CDI (the *Clostridium difficile* Infection ABM, or CDIABM) to examine fundamental dynamics of the pathogenesis of CDI and its response to treatment with anti-CDI antibiotics and a newer treatment therapy, Fecal Microbial Transplant (FMT). The CDIABM focuses on one specific mechanism of potential CDI suppression: commensal modulation of bile acid composition. Even given its abstraction, the CDIABM reproduces essential dynamics of CDI and its response to therapy, and identifies a paradoxical zone of behavior that provides insight into the role of intestinal nutritional status and the efficacy of anti-CDI therapies. It is hoped that this use case example of the CDIABM can demonstrate the usefulness of both agent-based modeling and the application of abstract functional representation as the biomedical community seeks to address the challenges of increasingly complex diseases with the goal of personalized medicine.

Introduction: Agent-based Models as Dynamic Knowledge Representations

Agent-based modeling is a discrete event, object oriented, rule based and often spatially explicit computer simulation method that represents systems as a series of interacting components (agents) [1-5]. An agent-based model (ABM) consists of populations of computational objects (or *agents*) corresponding to a specified organizational level of the

* To whom correspondence should be addressed: University of Chicago Department of Surgery 5841 South Maryland Ave MC 5094 S-032 Chicago, IL 60637 Phone: 773-702-9742 Fax: 773-702-6120 docgca@gmail.com.

system being studied. The *agent class* of an ABM represents a grouping of agents of a similar type, identified by shared properties and characteristics, and manifested by *agent rules*. ABM rules are often expressed as conditional statements (“if-then” statements), making ABM suited for representing mechanisms identified from basic science research. However the general nature of a “rule” allows other types of mathematical or computational models (i.e. differential equation, stochastic or network) to be used as rule systems [5-11]. Individual agents, while embodying the shared properties of their parent agent class, are able to manifest diverging behavioral paths based on differing inputs within a heterogeneous simulation environment, allowing an ABM to generate system level behaviors arising from sets of lower level behaviors. Agent-based modeling has been used in a wide range of applications, such as ecology [12], social science [13], economics [14] and epidemiology [15]. Agent-based modeling has also extensively applied to biomedical systems, representing and examining biological systems as driven by multi-cellular interactions in the study of sepsis [16-19] cancer [6, 20-23] cellular trafficking [24-28] and wound healing [10, 29, 30].

Given their rule-based nature, ABMs facilitate the representation of mechanisms and hypotheses present in the biomedical literature, allowing them to serve as dynamic knowledge representations of mechanistic hypotheses [18, 31]. ABMs are also intrinsically cross scales of biological organization, utilizing behavioral rules (Scale #1) to determine individual agent behavior (Scale #2) and then aggregating individuals into population dynamics of the global system (Scale #3). This ability to nest and compartmentalize different levels of biological behavior makes agent-based modeling well suited for creating scalable modular models [5, 6, 11, 19, 32].

ABMs are related to other spatially discrete modeling methods, most notably cellular automata, though the mobile capability ABM agents and ability to represent a wider range of system characteristics could allow cellular automata to be considered as a special type of ABM. Some of the properties of ABMs include:

1. Agent-based models (ABMs) have a *spatial geometry*. ABM agent behavior is driven by interactions defined by the communication and interaction network for each agent. This agent interaction neighborhood does not necessarily represent physical proximity but rather the configuration of some set of other agents with whom an agent can interact. This definition of an agent neighborhood is consistent with the bounded nature of the sense-and-respond and message passing capabilities of biological objects.
2. ABMs utilize *population effects*. Each ABM agent class has multiple computational instances that form a population of agents. Heterogeneous behaviors arising from the individual application of agent-rules produce population dynamics that are the system-level output of the ABM. A classic example of this phenomenon is the behavior of flocks of birds, in which simulations utilizing relatively simple interaction rules among birds can lead to sophisticated flocking patterns without an overall controller [33].

3. ABMs utilize *stochasticity*. Many biological systems have behaviors that appear to be random [34, 35]. Probabilities of a particular behavior can be determined for the population as a whole, and used to generate a probability function for the behavior of a single agent incorporated into the agent's rules. This stochasticity adds to the variation in individual agent behavior, and leads to greater agent population heterogeneity within a simulation run.
4. ABMs are *modular*. Agent rule sets are intrinsically modular, such that new information can be added either via the introduction of new agent-types or by the modification of existing agent rules without having to re-engineer the entire simulation. More generic cell types can be expanded to include a finer degree of detail resulting in sub-categories of cells, and new mediators can be added by creating new cellular-state or environmental variables and rules. Multiple ABMs can be aggregated, providing that their points of contact and interaction are consistent across the incorporated ABMs [18, 19].
5. ABMs facilitate *abstraction*. In the initial construction of an ABM it is advantageous to keep the rules as simple and verifiable as possible, often at the expense of mechanistic detail. What initially may seem to be a limitation is actually of considerable benefit, as ABMs representing incomplete and uncertain mechanisms can provide a mean of testing the plausibility of those mechanisms [12]. As with all computational models, the greater fidelity of mapping between the ABM and its biological counterparts enhances the correlation between simulation results and the real-world behaviors. An iterative process of refinement of an ABM will lead to increased detail, perhaps a stronger correlation to real-world data and a greater confidence in the ability of the ABM to describe observable phenomena [4, 18].

The advantages of agent-based modeling are most evident when trying to integrate multiple populations of sub-components (such as biological cells) that interact in a highly dynamic fashion. This is not only true of the internal physiology of multicellular organisms, but also characterizes the interactions between domains/kingdoms, such as between microbe and host, either in cases of invasive infection or ecological crosstalk.

Agent-based modeling of gastrointestinal infections

The intestinal tract is an example of such a setting, where populations of microbes interact with host cells to produce, at baseline conditions of health, a dynamically robust and synergistic ecosystem. Gastrointestinal infections can be modeled as either the introduction of a new agent class representing an invasive pathogen, or by the conversion of a previously commensal microbe into a virulent pathogenic form in response to alterations of the gut environment such as stress, nutrient depletion or the administration of antibiotics. Agent-based modeling has previously been used to examine intestinal-bacterial interactions/ infections ranging from upper GI tract *Helicobacter pylori* infection [36], to investigating the contribution of bacteria to necrotizing enterocolitis [37] to colonic interactions involving the pathogenesis of gut derived sepsis [38] and salmonella infection [39]. The first of these studies involved the use of an agent-based modeling platform specifically developed at the Virginia Bioinformatics Institute to study intestinal immunity [40], while the second two

studies were developed using the general purpose agent-based modeling toolkit NetLogo [41]. Not only can the introduction of pathogenic bacteria disrupt the healthy ecology present in the gut, but the application of systemic factors, such as drugs (most notably antibiotics), or alterations in nutrient intake (as would be seen in acutely ill patients) can result in severe disturbances to the baseline microbial populations with consequent pathophysiological effects. One of the most notable and clinically significant examples of this type of nosocomial infection is *Clostridium difficile* colitis/infection.

An Example ABM of Gastrointestinal Infection: Dynamic knowledge representation of *Clostridium difficile* infection (CDI)

C. difficile is a gram-positive bacillus that has both a spore and vegetative form, where the vegetative form produces exotoxins that lead to diarrhea and intestinal inflammation. *C. difficile* infection (CDI) is the most common nosocomial intestinal infection, and represents a significant source of morbidity in hospitalized patients [42-44]. The basic pathophysiology of CDI is recognized as being initiated by the administration of systemic antibiotics, which leads to a disruption of the commensal intestinal microbiome and allows the opportunistic rise of *C. difficile* bacteria [42]. However, the specific mechanisms by which commensal microbiota suppress CDI are still under investigation. Candidate mechanisms include: commensal modulation of intestinal bile acid metabolism, commensal production of bacterocins (anti-bacterial toxins produced by one species to suppress another), commensal modulation of host defenses and immune responsiveness (Reviewed in [42]). Traditional and standard attempts to reduce the spread of *C. difficile* in the healthcare setting focus on reducing the contamination of surfaces where the spores can persist, thereby reducing subsequent patient-to-patient transmission. However, recent microbial genetic studies of hospitalized patients with CDI have shown that patient-to-patient transmission of the pathogen is less frequent than previously thought [43], with the significant implication that many healthy individuals harbor sporulated or non-virulent *C. difficile* that under specific conditions, i.e. systemic antibiotics, can lead to blooms resulting in CDI.

The standard treatment for CDI is stopping the administration of broad-spectrum antibiotics and administering specific antibiotics targeting *C. difficile*, namely metronidazole or vancomycin. This strategy treats CDI as any other type of bacterial infection to be eradicated through antibiotics. However, despite the general efficacy of this approach, limitations in terms of development of antibiotic resistance in *C. difficile*, recurrent infections, refractory colitis and collateral resistance (i.e. the generation of vancomycin resistant Enterococcus) have lead to interest in alternative therapeutic strategies [45]. Given the recognition that CDI arises because of some loss of suppression achieved by the baseline commensal microbiome [42], there is interest in therapeutic strategies aimed at reconstituting the commensal microbiome, either through the administration of probiotics [46] or, more recently, fecal microbial transplants (FMT) [45]. FMT is the enteric administration of fecal contents obtained from healthy volunteers. The potential advantage of FMT over probiotics is that the microbial communities present in FMTs are considerably more complex, with a resulting population/ecological robustness that could more effectively lead to effective suppression of a *C. difficile* bloom [45].

However, despite the demonstrated efficacy of FMT and an intuitive rationale for why it works, there remain several important questions concerning the mechanisms of how FMT suppresses *C. difficile*. Different potential mechanisms of CDI suppression may have implications on the type of FMT used, the timing of FMT and potential adjunctive measures that could impact its efficacy [42]. One hypothesis of the mechanism of FMT centers on the known effects of bile acids on the germination and sporulation of *C. difficile*. Primary bile acids are produced in the liver and secreted into the bile, which then flows into the intestinal tract. Taurocholate, a primary bile acid derived from main precursor bile acid cholate, is known to induce the germination and growth of *C. difficile* [47, 48]. Commensal intestinal bacteria convert taurocholate to the secondary bile acid deoxycholate, which suppresses the growth of *C. difficile* and induces sporulation. Facilitating the conversion of the pro-growth taurocholate to the growth-suppressing deoxycholate provides a mechanism for the role of commensal bacteria in suppressing *C. difficile* growth (and therefore CDI) [47]. In addition, in states of health where potential patients are assumed to have an adequate oral intake, commensal microbes are noted to be more metabolically efficient than *C. difficile*, allowing them to out-compete germinated *C. difficile*, providing another means by which commensal microbes can suppress *C. difficile* [49].

We have created an agent-based model of CDI (the *Clostridium difficile* Infection Agent-based Model or CDIABM), to examine the ecological dynamics of this system, initially in a state of health, then in the pathogenesis of primary CDI secondary to nutrient depletion (associated with acute illness) and antibiotic administration, and then to characterize the efficacy of therapies including CDI-directed antibiotics and FMT. The CDIABM represents intestinal epithelial surfaces as the background environment for populations of abstractly general commensal microbes, and incorporates a generic representation of commensal microbes and *C. difficile* bacteria in both its vegetative and spore forms. Bile acid and nutrient flow are abstractly represented as environmental variables that influence, and are influenced by, the presence of the different types of microbes. The administration of antibiotics, both broad spectrum and specifically anti-CDI, are represented as culling functions that reduce targeted microbial populations. The application of FMT consists of repopulation of the virtual gut with a specified number of commensal microbes. Simulation experiments were performed examining the behavior of the system given different conditions such as varied nutrient levels, following the application of broad-spectrum antibiotics, and the treatment of CDI with either anti-CDI antibiotics or FMT.

We readily acknowledge that the CDIABM is an incomplete representation of the true complexity of the dynamics of CDI. Rather, we present this current version of the CDIABM as an example of the development and use of an ABM. We will note possible extensions of this model in the Discussion section. However, at a more fundamental scientific level, dealing with the incompleteness of knowledge is intrinsic to biomedical research [50], and we assert that a primary benefit and utility for the use of agent-based modeling is for dynamic knowledge representation in order to examine the consequences of a particular hypothesis and especially to identify unexpected and nonintuitive behaviors that may arise [19, 31, 51]. In order to foster the use of ABMs as a means of exploring potential hypotheses, we make the CDIABM available for download at http://bionetgen.org/SCAI-wiki/index.php/Main_Page.

Methods

Model Overview

The CDIABM was developed using the processes of dynamic knowledge representation [19, 31, 51] and the iterative refinement protocol (described in [4, 18]). The CDIABM was implemented in NetLogo, a freeware agent-based modeling toolkit [41]. The CDIABM is an abstract representation of a section of the gut, populated by different microbial populations (generic commensal microbes and both vegetative forms and spores of *C. difficile*), and containing various levels of luminal compounds (nutrients, bile acid species and *C. difficile* exotoxins). The overall intestinal surface is discretized by use of the grid, but within each grid the concentration of the luminal compound is represented homogeneously (this is a “patch variable” in NetLogo terminology). Luminal compounds are represented by different variables present per grid space. Commensal microbes and vegetative and spore forms of *C. difficile* are represented by mobile agents. The rules for each agent class are noted below. Rules often take the form of conditional statements, such as:

“If compound X is present, then activate receptor Y.”

Rules can operate at different levels of mechanistic detail; CDIABM rules are expressed as either Boolean logical or arithmetical statements. Figure 1 is an overview schematic of the primary components and interactions represented in the CDIABM. The intestinal environment (composed of luminal compounds and lining epithelial cells) plays host to populations of commensal microbes as well as both spore and germinated forms of *C. difficile*. The primary bile acid Taurocholic acid (TCA) is introduced into this environment by hepatic input. In the presence of commensal microbes it is converted to the secondary bile acid Deoxycholic Acid (DCA). If the environment has enough DCA and low enough levels of TCA, these conditions will prevent any *C. difficile* spores from germinating. If, however, the commensal microbial population is depleted through the administration of antibiotics, then this will allow the spores to convert to germinated *C. difficile*. The germinated *C. difficile* produce toxin, which damages the epithelial cells and releases a previously unavailable reservoir of resource, and have increased motility. Using these virulence capabilities, the germinated *C. difficile* now compete against the recovering commensal microbes, which can be augmented by administering a FMT. See Figure 2 for screenshots of the CDIABM demonstrating the baseline healthy gut, as well as CDI in multiple screenshots showing the spatial distribution of different layered environmental variables.

A Note on Parameters

As with all computational models, ABMs require the use of multiple parameters (constants utilized in the model's rules). For example, in the sample rule provided above, what this would actually look like in its implementation would be:

“If [some value of] compound X is present, then activate receptor Y [to some degree].”

It should be immediately evident that the behavior of any model is heavily dependent upon the parameters chosen, therefore the process of parameter selection is extremely important.

On one hand, choosing of parameters that “fit” experimentally available data substantially enhance the believability of a computational model (assuming it behaves plausibly with those parameters!). On the other hand, the actual acquisition of specific parameters is often extremely difficult, and actually often impossible given current experimental and sampling technologies. This latter condition, in fact, has substantially limited the adoption of computational models in biomedical research; a very stringent and restrictive criteria for what constitutes a “believable model” that is dependent upon quantitative parameter and model behavior matching substantially reduces the number of “believable models” that can actually be produced. In fact, it can be argued that the ability to obtain such specific parameters can only be obtained in highly constrained and artificial experimental conditions, with the end result of a model “valid” for its experimental conditions, but of little applicability beyond those conditions [4].

This demonstration of agent-based modeling takes a very different view. As noted above, one of the advantages of agent-based modeling is its embracement of abstraction as a means of dealing with incomplete knowledge. By utilizing population effects as their primary output metrics, ABMs allow the characterization of system behavior in a more qualitative fashion, at least at first. For this reason, Pattern Oriented Analysis [12] and the use of Face Validity [52] (both discussed in a bit more detail below) are heavily utilized in the interpretation of ABMs. This shifts the utility of dynamic computational modeling from quantitative prediction or engineering to explorations of plausible and recognizable behaviors.

This type of modeling goal has an effect on the choice of parameters used in our ABM example. As can be seen in the sample rule above, ABM rules start off as logical statements; the addition of conditional modifiers turns these rules into semi-logical statements closer to arithmetic. This allows certain types of parameters, specifically those associated with processes with known time scales, to be derived arithmetically. For example, the turnover rate of intestinal epithelial cells is known to be 3-5 days. Given that each time-step of the CDIABM is 1 minute, working backwards allows the calculation of an epithelial regeneration rate of 0.02 “health points” to recover the baseline healthy level of 100. Note that the creation of “health” as a scalar metric is an abstraction; it does not directly correlate to a specific quantifiable measurable value, but does satisfy (potentially) an intuitive sense of appropriateness. There are also other sets of parameters that are even more abstract. For instance, the conversion and metabolism rates for the various microbial species in the CDIABM are heavily abstracted into unitless values. Even though these rates are potentially extractable and knowable, within the context of the system being represented, their actual values are not important. Rather, it is the relationship they have to other related parameters that is crucial for determining the behavior of the model. The relative dependencies of these types of parameters can prove very challenging if one required quantitative fidelity, but given the modeling goal of determining and examining sets of overall system behaviors, this type of parameter representation is appropriate (and arguably more relevant to translating the findings of a model beyond its specific implementation). As such, the establishment of these parameter values often starts with an arbitrary range, and sometimes followed by a heuristic, hand-fitting process involving repeated runs of the model and adjustment based on plausible behavior. If such plausible behavior cannot be generated, then this points to a

fundamental insufficiency in the model. In the sections below, we will specifically note when parameter selection is based on some known overall behavior, when heuristic fitting is applied or arbitrarily set.

We make this extensive digression because we believe that having foreknowledge of what can possibly be expected from a computational model is absolutely vital to its utility, and necessary to avoid over-reach in interpreting its behavior and results.

Components of the Model

In order to reduce ambiguity, the names of agent-types and variables utilized in the CDIABM will be noted in Courier font, in order to distinguish them from their corresponding biological entities.

World

The intestinal surface is represented as a 2-dimensional square grid with edges that wrap, making it topologically a torus. Each grid space is populated by a single gut epithelial cell agent (Epi-cell). The total size of the CDIABM is 31 x 31 grid spaces, giving a total population of Epi-cells = 961. The size of the world was arbitrarily set.

Patch/Environmental Variables representing intestinal contents

Compounds that are present free in the intestinal lumen are represented as patch/environmental variables. The values of these variables represent the amount of the respective compounds present on a single grid space; diffusion of these compounds into surrounding grid spaces is accomplished using the “diffusion” function present in NetLogo. There are three such compounds represented in the CDIABM:

Nutrients—This variable generically represents the amount of nutrients present in the intestinal lumen, and represents the energy source for the resident microbes. The flow of nutrients through the gut lumen is modeled by the addition of nutrients per time step based on the nutrient-replenish-rate, with values ranging from 0.1 to 1.0 (unit-less and arbitrary range), where 1.0 represents a normal level of oral intake in a state of health. Reduction of the nutrient-replenish-rate represents the decreased oral intake associated with acute illness, a situation common to the hospitalized patients at risk for developing CDI.

Bile Acids—Primary bile acids are produced in the liver and excreted into the bile that flows into the intestinal tract (parameter values with arbitrary range). Primary bile acids, which are derived from the primary bile acid cholate, are represented by a single species in the CDIABM, taurocholate, which is known to induce germination and growth of *C. difficile* [47, 48]. Secondary bile acids, represented by the single type deoxycholate in the CDIABM, are produced by conversion of taurocholate by agents representing commensal bacteria (commensals)(conversion rate heuristically fit). Deoxycholate inhibits germination of *C. difficile* and induces sporulation [47].

Toxin—Germinated *C. difficile* produce two types of exotoxin (Toxin A and Toxin B) that both damage epithelial cells, leading to increased permeability, induction of inflammation

and eventual cell death [44]. The relative role and contribution of each of these toxins to the pathogenicity of *C. difficile* is unclear [44], and in the CDIABM they are aggregated into a single variable, toxin. The effect of the toxin is to damage the epithelial cell agents (epi-cells), providing both a marker of total system damage as well as releasing additional nutrients into the environment (see below). All parameters related to toxin production and effect heuristically fit.

Agent classes

Epi-cells—These are stationary agents that represent the epithelial cells composing the mucosal surface of the intestine. In the CDIABM the normal 3-dimensional intestinal topology of crypts and villi is abstracted into a flat 2-dimensional surface. Given the emphasis of the CDIABM on examining microbial population dynamics, represented epithelial cells serve only as a potential source of nutrients for the microbes if damaged by toxins. Epithelial cell growth and differentiation are reflected by a basal replication rate (simulating epithelial stem cell division) and an abstracted version of differentiation. Therefore, each Epi-cell agent actually represents the aggregated population of epithelial cells residing on a discrete section of intestine represented by a grid space. The overall health of the system is measured by the amount of damage acquired by the total Epi-cell population, the maximum amount being 96100 (given the 31×31 dimensions of the world grid, and a maximum health value of 100 per Epi-cell agent). Epi-cells are damaged as a result of exposure to toxin. When this occurs they leak/release nutrient into the surrounding environment consistent with the damaging effect of both Toxin A and Toxin B [44]. Epi-cells therefore represent a reservoir of potential resource that can be accessed by the virulent germinated form of *C. difficile*. Damaged epi-cells recover by restoring their health at a rate of 0.02/step, such that complete repair occurs at ~3-4 days simulated time.

General Approach to modeling microbes

Agents representing microbes have sets of rules abstractly representing metabolism and growth, including uptake of nutrients to maintain internal energy levels, energy expenditure (directed to either growth, movement, or production of toxin) and a reproduction energy threshold. Microbial agents have a basal-metabolism-rate (arbitrary range), representing energy expenditure to maintain baseline internal functions, a nutrient-uptake-rate (heuristic fit), representing their ability to extract nutrients from the environment, and a replication-threshold (heuristic fit), representing the point at which enough internal energy has been stored to allow division. The metabolism rules provide for uptake of nutrients, and energy expenditure for defined cellular functions: any residual amount at the end of a time step is stored as an energy reserve that aggregates towards the replication-threshold. When nutrient uptake is insufficient to meet basal metabolic requirements there is a net loss of energy per time step; if this persists then eventually the microbial agent's energy reserves fall to 0 and the microbial agent dies (or, as in the case of *C. difficile*, has a percentage chance of converting to a spore as described below). This abstracted life-cycle/metabolic utilization allows microbial agent populations to fluctuate based on nutrient availability (reflected by the nutrient-replenish-rate) (independent experimental variable). During initialization, baseline conditions for a particular nutrient-replenish-rate will lead to microbes will having

a period of exponential growth until the nutrient carrying capacity of the system is reached, after which microbial populations maintain a steady state level.

Commensals—Commensal microbes are assumed to be optimized to maximizing their biomass, and as a result they have a higher efficiency in converting available nutrients towards growth [49]. One manifestation of this growth optimization is reflected by their lack of motility (which would represent an additional source of energy expenditure). As a result the commensal population spreads only by division/replication. In addition to their growth properties, commensals have a fixed rate of converting taurocholate present on their current patch into deoxycholate.

C.difficile—*C. difficile* bacteria can exist in two forms, each of which represents the two ends of the spectrum of bacterial response to nutrient scarcity and stress. The germinated form represents a high-risk, high-reward strategy where there are increased basal expenditures manifesting in the form of increased motility (swarming movement to seek out nutrients) and the production of toxin (to make available additional nutrient resources). The *C.difficile*_spore form, on the other hand, represents a dormancy strategy where metabolic pathways are shut down to reduce basal energy requirements to a minimum, resulting in loss of motility, increased environmental hardiness and resistance to the application of anti-*C. difficile* antibiotics. *C.difficile*_germinated forms have a higher energy expenditure rate than commensals per step based on their need to expend energy for motility and toxin producing functions. When faced with nutrient scarcity that leads to an ongoing negative energy balance per step, when their energy level drops below the sporulation-threshold there is a 15% chance that they will form spores as opposed to dying [53].

Simulation Experiments

The simulations carried out here using CDIABM are geared towards demonstrating plausibility of the hypotheses and mechanisms underlying the model. This is accomplished by having the model fit some set of defined qualitative pattern/phenotype present in the real world reference system. This approach is called Pattern Oriented Modeling [12], and is very commonly used in calibrating and validating ABMs. Initial validation of an ABM is accomplished when there is satisfactory matching between the ABM and its referent with parameter values that are not clearly implausible, a level of validation is termed *face validation* [52]. In short, a model is determined to be face valid if its behaviour is subjectively determined to be “reasonable.” [52]

While being the most basic level of validation possible for a simulation, establishing face validity is of extreme importance in the use of computational models for dynamic knowledge representation of biomedical systems. This is because biomedical research is primarily a discovery-oriented endeavour, where the primary procedural challenge is being able to separate plausible from implausible hypotheses [50, 54]; this being the case, the more rigorous levels of validation often required in engineering-grade simulations are not simply not possible due to the fact that the behavioural properties and rules of the system are not sufficiently known (see Discussion).

A run of the CDIABM consists of multiple iterations of the list of agent actions, with each time step = 1 minute of simulation time. Each simulation experiment consists of multiple runs of the CDIABM executed for 21 days simulated time. Given the stochasticity inherent in the execution of the CDIABM, each run is analogous to a distinct individual, such as would be present in a laboratory experiment or a clinical trial. Simulation experiments included:

1. Parameter Sweep of nutrient-replenish-rate: The nutrient-replenish-rate represents the intake of food into the intestinal tract; lower levels of nutrient-replenish-rate are intended to reflect the decreased oral intake seen in hospitalized patients at risk for CDI. For this reason, we consider the value of nutrient-replenish-rate as a proxy for the overall health status of the individual. However, since restriction of oral intake alone is not a recognized cause of CDI, the identification of such a threshold would place a lower limit on the set of parameter values that could be reliably used in subsequent simulation experiments on CDI.
2. The generation of primary CDI through the application of broad-spectrum antibiotics. The application of broad-spectrum antibiotics, the recognized initiating factor for CDI, was modeled as a culling function that eliminated 80% of commensal bacteria every 6 hours for the entire period of the simulation run (21 days).
3. Treatment of CDI with cessation of broad-spectrum antibiotics. Broad-spectrum antibiotics (80% efficacy) were administered every 6 hours for 72 hours and then stopped. Simulation runs were continued to evaluate recovery at 21 days.
4. Treatment of CDI by cessation of broad spectrum antibiotics and the administration of anti-CDI antibiotics, both prior to and after development of antibiotic resistance. Broad-spectrum antibiotics were administered every 6 hours for 72 hours, then stopped and followed by the application of an anti-CDI antibiotic every 6 hours for 14 days. An anti-DCI antibiotic as a culling function of 80% represented a naïve CDI, while an anti-CDI antibiotic with a culling function of 50% represented a CDI with some development of antibiotic resistance, a condition more and more commonly seen. Simulations were continued to evaluate system state at 21 days.
5. Treatment of CDI with FMT. CDI was induced by the application of broad-spectrum antibiotics every 6 hours for 72 hours, and then followed by a single dose FMT consisting of repopulation of the CDIABM with the baseline level of commensals (= 3000). Simulations were continued to evaluate outcome at 21 days.

Simulation experimental conditions #2 -5 were all run across a range of nutrient-replenish-rates (0.3/patch/step to 1.0/patch/step), with $n = 100$ simulation runs per condition, where each individual simulation utilized a different seed for the random number generator, resulting in a stochastic or random variance in the simulation's behavior for each different random number seed. As noted above, this property of ABMs allows each individual run to be treated as an individual experimental object. Output metrics included: 1) Total-system-damage, reflected by the total amount of damage inflicted on the epi-cell population and representing the severity of the CDI, 2) Total count of commensals, reflecting the degree of

restitution of the baseline microbiota, 3) Count of *C.difficile*_total, which includes both germinated and spore forms, reflecting the efficacy in suppression/elimination in each condition, and 4) Count of *C.difficile*_germinated, reflecting active CDI, and 5) Count of *C.difficile*_spores, reflecting latent, potentially recurrent CDI.

Results

A parameter sweep of nutrient-replenish-rate from 0.1/patch/step to 1.0/patch/step demonstrated this lower threshold to be 0.3/patch/step, below which there was spontaneous generation of a CDI. This threshold represents a point where there is a competitive advantage for the *C.diff*_germinateds resulting from their virulence factors (toxin production and motility) that leads to a perpetuating CDI. This represents an implausible condition to arise solely from nutrient restriction; therefore, all subsequent simulation experiments utilized a range of nutrient-replenish-rate from 0.3/patch/step to 1.0/patch/step. However, as subsequent simulation experiments will show, the existence of the tipping point in competitive reproductive fitness from commensals to *C.diff*_germinateds does play a role in the response curves in CDI and its treatment.

The results of subsequent simulations are demonstrated in Figures 3-7. Plots compare mean values ($n = 100$) for each of the different simulation experimental conditions against each other based on the selected output metric.

The generation of primary CDI through continued application of broad-spectrum antibiotics leads to maximal total-system-damage across the entire range of nutrient-replenish-rate (Figure 3). Recognizing that this is not a clinically relevant condition, these simulations nonetheless demonstrate that the CDIABM gives plausible behavior that is bounded at the upper end of disease behavior without an unexpected spontaneous recovery in the face of continued commensal culling.

In terms of spontaneous recovery from primary CDI following cessation of broad-spectrum antibiotics (an accepted therapeutic strategy for less severe CDI) there was a threshold effect between nutrient-replenish-rate of 0.6/patch/step and 0.65/patch/step (Figure 3). At 0.6/patch/step and below there was near sustained CDI, manifest by high total-system-damage (Figure 3), decreased commensals (Figure 4) and high levels of total and germinated *C. difficile* (Figure 5 and 6), whereas at >0.65/patch/step there was progressive recovery of total-system-damage to near complete recoverability at 0.85/patch/step and greater. Commensal populations started to recover in a similar pattern (Figure 4), with evidence of suppression of *C.diff*_germinated (Figure 6) but with a concurrent increase in residual *C.diff*_spores (Figure 7). This set of transitions likely reflects the tipping point between favored reproductive fitness between *C.diff*_germinateds and commensals, and is consistent with the mechanism of CDI suppression based on alterations of bile salt composition by the commensals.

Anti-CDI antibiotics of 80% efficacy were effective in suppressing and treating CDI, with an expected decreased effect with the anti-CDI-antibiotics at 50% efficacy (Figures 3 and 5). Both reduced levels of *C.diff*_germinated and *C.diff*_spores, but there was a paradoxical

increase in the degree of induced damage/CDI between nutrient-replenish-rates of 0.5 to 0.85, peaking at 0.65 for both types of anti-CDI antibiotics (Figure 3). We hypothesize that in this range of nutrient-replenish-rate the resource distribution density favor an expanded range of the *C.diff_germinated* damaging effect due to both its motility capability and toxin effects, since the overall population levels of *C.diff_germinated* are not elevated in this range (Figures 5 and 6). This suggests that while eventually effective, eradication strategies with anti-CDI antibiotics led to an interval where *C.diff_germinated* population culling actually led to an increase spatial distribution of *C.diff_germinated* and its produced toxin with the consequence of worsening CDI.

As would be expected, the application and efficacy of FMT is affected more directly by the nutrient-replenish-rate, with improved system damage (Figures 3), number of commensals (Figure 4) and suppression of *C.difficile_total* (Figure 5) and *C.diff_germinated* (Figure 6) by conversion to *C. diff_spores* (Figure 7). The reason for this pattern is the enhanced growth of commensals in more resource rich environments, allowing the more rapid recovery of commensal populations to a level able to provide adequate suppression. However, given their function of converting taurocholate to deoxycholate, which in turn converts *C.diff_germinated* to *C.diff_spores*, *C.diff_spore* levels remained higher than those seen with administration of anti-CDI antibiotics at lower nutrient-replenish-rates (Figure 7). This is likely due to smaller overall commensal population numbers due to limitations on carrying capacity, with consequently less effective suppression. The residual *C.diff_spore* population in these conditions places those individuals at risk for recurrent CDI should broad-spectrum antibiotics be re-administered in the future.

Discussion

Agent-based modeling is an integrative modeling framework that can readily be used for communicable dynamic knowledge representation [18, 19, 31, 55]. As knowledge-based models constructed by implementing bottom-up mechanisms (as opposed to inductive models, where mechanisms are inferred with the goal of explaining data), ABMs address different modeling questions than equation-based inductive models. ABMs are not really a form of “data analysis,” readily developed directly from a mass of raw data. Rather, they require that the researcher have a mechanistic hypothesis that, when expressed and implemented in an ABM, can be used to generate simulated data/behavior. This output behavior is then compared to the real-world data set in an ongoing process of validation intended to increase trust in the model. Agent-based modeling, because of its emphasis on “things doing things”, is often more intuitive for biomedical researchers than more formal mathematical modeling, providing a lower threshold barrier for researchers to instantiate their conceptual models and integrate *in silico* methods with traditional *in vitro* and *in vivo* experiments [55]. This facilitates an overall iterative process where inductive models are applied to large data sets, wet lab experiments are carried out to investigate the mechanisms inferred from the inductive model, and the experimentally confirmed mechanisms are used as a basis of an ABM which would close the discovery loop by recapitulating the original data set [50, 54].

In keeping with this concept of iterative dynamic knowledge representation, the CDIABM can be considered a “cartoon” of specific aspects of CDI, and as such is not expected to provide any insight into more detailed processes not currently included, such as direct inter-microbe interactions, host epithelial or immune biology or biochemical properties of *C. difficile* toxins. All these limitations represent potential avenues for expansion of the existing model. Rather, for the current model, the goal is to determine whether, given specific known biological processes, when those processes are instantiated to demonstrate their dynamic consequences, there are unexpected and unanticipated behaviors identified. This is a fundamental scientific step that is poorly performed in biology, due in part to the complex interactions present in biological systems and also due to the general lack of formal, theoretical analysis of biological systems [50]. In the specific example of the CDIABM, the unanticipated behavior is noted as the paradoxical increase in total-system-damage between nutrient-replenish-rates of 0.5/patch/step and 0.85/patch/step when treating a CDI with antibiotics. This is associated with a transition point where FMT efficacy improves to being greater than antibiotic therapy. This finding suggests that there is a nonlinear relationship between the nutritional resources available for the enteric microbiota and the concurrent dynamics of the biota in terms of host consequences of CDI, a state space of CDI not previously recognized. The potential clinical consequence of recognizing these zones of system behavior would be further refining of appropriate nutritional status conditions for either traditional anti-*C. difficile* antibiotics, FMT, or some combination of both, or indication for augmented directed nutrient supplementation during treatment. All these steps move further in the desired direction of more personalized medicine.

As with all computational models, the issue of validation is critical to the use and interpretation of agent-based models. In fact, the problem of validation is ubiquitous at nearly all phases of the drug development pipeline and biomedical research, a fact that has recently received much greater attention in terms of evaluating the validity of murine models of human disease [56, 57]. To a great degree, these publications represent a growing recognition that establishing the mapping between computational models and their underlying mechanistic hypotheses may be much more clear and explicit than having to try and account for the effects of the opaque complexity of an intact biological organism. As we have previously noted, the concept of qualitative pattern matching and face validity plays a vital role in evaluating the plausibility of ABMs [12, 31, 52]. Given the intrinsic incompleteness of all biomedical hypotheses, there is naturally a progressive approach towards establishing additional metrics for comparison as more features are added into the model; this process has been described by Hunt and colleagues in their Iterative Refinement Protocol [4, 18]. For the CDIABM, the addition of features such as greater representation of the epithelial and immune biology would then require the ability to access and retrieve corresponding information and data from these corresponding tissues in order to establish additional face validity of the resultant model. A key point here is that the addition of model detail must repetitively and similarly be subjected to qualitative face validity: it must be able to reproduce existing behavior in a plausible fashion before an attempt (if any) is made to obtain greater quantitative matching. If appropriate data is available, an increased degree of quantitative fitting can be attempted; in this fashion computational models can iteratively and progressively aspire to greater fidelity and “engineering-grade” validation. However,

practitioners should be aware that there is a persistent danger that the attempt to “improve” quantitative fitting in order to obtain more rigorous, engineering-grade validation (which, at the current level of experimental technology, requires the use of highly constrained and reductionist experimental biological models in order to obtain such quantitative data) will reduce the ability of the resultant computational model to capture a more comprehensive depiction of a complicated reference system (i.e. clinical relevance). It should be noted that this “mapping problem” is pervasive throughout biomedical research, i.e. how similar actually are preclinical models to their intended clinical disease targets [56, 57]? Because of this danger, as well as the “discovery” oriented goal of much biomedical research, we have suggested that the power of modeling is accentuated by this detailed, qualitative approach as opposed to attempting to focus on creating models with a narrow quantitative fit [51, 58]. This is particularly true in ABMs where the addition of detail often acts as an additional constraint on the behavior of the resulting model (as opposed to systems of differential equations, where the addition of equations can often lead to an increase in the degrees of freedom in the expanded model. This recognition is of critical importance in being able to appropriately use ABMs within the context of biomedical research, that they should be considered as useful steps within a general investigatory process and not end-product targets in of themselves. Then, as experimental technology improves to better map/match experimental platforms to the more complicated clinical setting, or sampling technology improves to a point where such data can be directly extracted from the clinical population, greater quantitative validation can be applied to produce higher fidelity models.

Meeting the challenges of the Translational Dilemma by enhancing the transition of basic mechanistic knowledge into effective therapeutics will require an expansion of the set of scientific methods and tools [54]. Agent-based modeling, by being able to represent and address fundamental functional questions present in multi-cellular systems, can serve as a vital adjunct to data-driven informatics methods and traditional experimental processes. Perhaps most importantly, the efficacious use of abstract ABMs may help the biomedical sciences begin to gain recognition of the importance of abstraction and theory long present in the physical sciences and quantitative fields [50].

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Figure 1.

Overall schematic of the components and interactions in the CDIABM. The intestinal environment (composed of luminal compounds and lining epithelial cells) contains populations of commensal microbes and both spore and germinated forms of *C. difficile*. The primary bile acid Taurocholic acid (TCA) is introduced into this environment by hepatic bile flow. Commensal microbes convert TCA to the secondary bile acid Deoxycholic Acid (DCA). The presence of DCA and absence of TCA prevent any *C. difficile* spores from germinating. Depletion of the commensal microbial population by the administration of antibiotics, allows the spores to convert to germinated *C. difficile*. The germinated *C. difficile* produce toxin, which damages the epithelial cells and releases a previously unavailable reservoir of resource, and have increased motility. The germinated *C. difficile* now compete against the recovering commensal microbes, which can be augmented by administering a FMT.

Figure 2.

Screenshots of the CDIABM. Letter A: Screenshot of the CDIABM at a baseline healthy state. Epi-cells (pink circles) are placed on the world grid, the intensity of the pink color reflects their health status. Commensals (green bugs) can be seen over the epi-cells. C.Diff_spores (white circles) can be seen sparsely scattered across the surface. Letter B: Zoomed in area of CDI following administration of broad-spectrum antibiotics. Damaged epi-cells are seen as darkened circles. Commensals have been depleted and C.diff_germinateds (yellow bugs) can be seen over damaged epi-cells. Letter C: Zoomed in area of CDI showing levels of taurocholate (higher intensity blue-green areas denote greater levels) that has not been converted to deoxycholate due to the depletion of commensals. Note that these areas contain the C.diff_germinateds. Areas where commensals remain have a darker background, denoting areas with lower taurocholate that has been converted to deoxycholate. Letter D: Zoomed in area of CDI showing levels of deoxycholate (higher intensity orange areas denote greater levels). This distribution is the reverse of Letter C, and represents areas of remaining commensal activity. Letter E: Zoomed in area of CDI showing levels of toxin (higher intensity green background denote greater levels) produced by C.diff_germinateds. These areas correspond to damaged epi-cells.

Figure 3.

Average values of total-system-damage ($n = 100$) for all conditions across parameter sweep of nutrient-replenish-rate. This graph depicts the severity of CDI as represented by damage to epi-cells. “Primary CDI” occurs when broad-spectrum antibiotics are applied for the entire duration of the simulation run; the damage to the system remains maximal for all values of nutrient-replenish-rate. “CDI Stop Ab Only” demonstrates that for low nutrient-replenish-rates CDI is maximally severe, though there is a transition to less severe CDI as nutrient-replenish-rate increases > 0.60 /patch/step, consistent with a recovery of commensals due to increased nutrient availability (See Figure 3). Anti-CDI antibiotic therapy is effective in decreasing the severity of CDI, with more pronounced with the more efficacious (80% Eff) anti-CDI antibiotics. However, there is a paradoxical increase in the severity of CDI in both anti-CDI antibiotic groups between nutrient-replenish-rate of 0.5 /patch/step and 0.85 /patch/step. “FMT” becomes more effective with increasing nutrient-replenish-rate, due to facilitating increased growth and recovery of depleted commensals. At higher levels of nutrient-replenish-rate (> 0.65 /patch/step) “FMT” becomes more effective than anti-CDI antibiotics.

Figure 4.

Average values of counts of commensals ($n = 100$) in all conditions across a parameter sweep of nutrient-replenish-rate. This graph demonstrates that commensals in nearly all conditions recover in a linear fashion with increasing nutrient-replenish-rate. The exception is for “CDI Stop Abs Only,” where there is discontinuous jump to a higher recovered population at the transition from nutrient-replenish-rate of 0.6/patch/step to 0.65/patch/step. There is a tipping point in the reproductive fitness between commensals and *C.diff_germinateds*, where at <0.6 /patch/step the virulence factors of *C.diff_germinateds* (toxin production and motility) are reproductively favored, and it can be expected that the CDI becomes self sustaining (also see Figure 4).

Figure 5. Average values of counts of total *C. difficile* (*C.diff_germinateds* plus *C.diff_spores*) ($n = 100$) across a parameter sweep of nutrient-replenish-rate. Anti-CDI antibiotics and FMT show expected reduction of total *C. difficile* agents. The discontinuity seen in “CDI Stop Abs Only” at nutrient-replenish-rate 0.6-0.65/patch/step again demonstrates the transition between relative reproductive fitness between *C.diff_germinateds* and commensals (also see Figure 3).

Figure 6.

Average values of counts of C.diff_germinateds ($n = 100$) across a parameter sweep of nutrient-replenish-rate. This graph demonstrates that the majority contribution to total C.difficile agents is made by C.diff_germinateds. Note that there is not a significant increase in the end population of C.diff_germinated in either of the anti-CDI antibiotic treatment groups, despite greater total-system-damage in the range of nutrient-replenish-rate from 0.5-0.85/patch/step. This suggests that while eventually effective, eradication strategies with anti-CDI antibiotics led to an interval where C.diff_germinated population culling actually led to at least an increase spatial distribution of C.diff_germinated and its produced toxin. Alternatively, the response of C.diff_germinateds to “FMT” demonstrated a nutrient-dependent effect on increasing commensal reproductive fitness, leading to suppression of C.diff_germinateds by bile acid conversion.

Figure 7.

Average values of counts of C.diff-spores ($n = 100$) across a parameter sweep of nutrient-replenish-rate. The most notable findings from this plot are the greater residual numbers of C.diff_spores following “FMT” compared to anti-CDI antibiotics for nutrient-replenish-rates < 0.65 /patch/step. This is also the transition point seen in the relative efficacy of “FMT” versus eradication via anti-CDI antibiotics. The high residual C.diff_spore levels suggest increased risk of CDI should subsequent broad-spectrum antibiotics be required again.