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A cellular automaton model to find the risk of developing autism through gut-mediated effects

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ABSTRACT

Background: One of the risk factors for the development of Autism Spectrum Disorder (ASD) is hypothesized to be an imbalance in the gut microbiome. Alterations in the relative numbers of gut microbiota may contribute to such a disruption in normal bacterial diversity. It is assumed that this process may be adequately mirrored for the purpose of the current paper by modeling the dynamic shifts in the numbers of three bacterial species, namely *Clostridium, Desulfovibrio*, and *Bifidobacterium*. Such imbalances in the gut microbiome are thought to promote the development of increased gut permeability (the so-called "leaky gut") which in turn is a potential risk factor for the development of ASD.

Methods: We constructed a mathematical model using 2-D Cellular Automata to simulate the growth rates and interactions of three bacterial species, namely *Bifidobacterium, Clostridium* and *Desulfovibrio*, with each other and with available nutrients in the gut, and particularly following the introduction of *lysozyme* into the gut.

Results: It was observed from the modeled simulation that increasing or decreasing the population of *Clostridium* in the gut produces key shifts in the gut microbiome which could potentially increase or decrease the risk of ASD.

Conclusion: Simulations using our cellular automaton model suggest that it could be useful in predicting the effects produced by alterations to key components of the gut microbiome. In particular, the model demonstrated that the introduction of *lysozyme* in the gut results in steep reductions in *Clostridium* growth rate, which in turn could potentially alter the gut microbiome population in such a way as to significantly reduce the risk of developing ASD.

1. Introduction

Autism spectrum disorders (ASD) are complex disorders linked to dysfunctional neurodevelopment, leading to poor social interactions and communication, restricted patterns of interest, and repetitive or stereotyped behaviors [1]. The current incidence of ASD is reported to be 1 in 68, according to the Centers for Disease Control statistics (2014) [2]. While ASD has been the focus of many research studies, its causes are still not known [3].

Children with ASD often also have disorders of the gut, such as diarrhea, constipation, bloating, and gastro-esophageal reflux [4,5]. The exclusion of casein or gluten from the diet, or treatment with vancomycin, has been reported by some investigators to result in some improvement in ASD symptoms [6,7]. These findings have prompted intensive research to identify the possible contributions of a change in the gut microbiota to ASD pathogenesis [5,8–14].

The human gut microbiome comprises 10^{14} microbes, about one kilogram in all, which include over a thousand different species. These take part in the digestion and fermentation of dietary components that remain undigested in the upper gastrointestinal tract. Even in the normal host, the gut microbiota contributes to antimicrobial protection and immune modulation as well as maturation of the gastrointestinal tract.

Four phyla account for over 90% of the healthy gut microbiome in adults, namely, *Bacteroidetes* including the *Prevotella* genus, *Firmicutes* including *Lactobacillus* and *Clostridia*, *Proteobacter* including *Enterobacter*, and *Actinobacteria* including *Bifidobacterium* [15,16].

The chief contributors to the composition of the gut microbiome include genetic, age-related and dietary factors [17–20]. Multiple studies have linked inflammatory bowel disease, obesity and several allergic conditions to changes in the gut microbiota [21,22]. The term "gut-brain-microbiota axis" refers to the multiple close interactions between these systems, with many of the resulting communications being mediated by the vagus nerve [23].

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In ASD, an alteration in the relative numbers of various phyla has been repeatedly identified. Some researchers report a reduction in *Firmicutes* and an increase in the number of *Bacteroidetes* [24]. However, others have found an increase in the relative abundance of genera like *Clostridia, Caloramator, Alistipes, Sarcina, Akkermansia, Lactobacillus, Enterobacteriaceae* and *Sutterellaceae* on the one hand [15,24–31], while genera such as *Bifidobacteria, Desulfovibrio, Coprococcus, Veillonellaceae* and *Prevotella* are lower in number than average, on the other [24,32]. In view of the complexity of the human gut ecology, such divergence is not surprising. Overall, however, the pathogenetic mechanism is thought to be related to the overgrowth of certain species like *Clostridium*, in particular, quite commonly following the use of antibiotics which inhibit the growth and proliferation of other normal components of the gut microbiome [10,33,34]. This could potentially cause an overproduction of bacterial toxins which could result in ASD [35–38].

In children with ASD, the levels of volatile organic compounds (VOCs), certain short-chain fatty acids (SCFAs), phenol derivatives and other metabolites have been found to be abnormal, potentially contributing to the severity and manifestations of this disease [35,37,39–46]. The reason for this kind of imbalance in the metabolome could be a change in the gut microbiota, especially a relative abundance of *Clostridium* species [8,47,48].

The outcome is hypothesized to be local inflammation and abnormal increases in gut permeability, which allows bacterial toxins and pro-inflammatory cytokines to reach the brain, producing ASD [49].

In view of this, researchers have focused on the role of dietary manipulations, including prebiotics and probiotics, as well as fecal transplants and microbiota transplants, to alleviate the clinical features of ASD. One case study reported a significant improvement in social affect as well as constipation following the administration of a ten-strain probiotic over four weeks to a child with ASD. Others have suggested a role for specific probiotics in ASD, but definitive evidence is still awaited [49].

While many diverse species are probably involved in the pathogenesis of ASD, some researchers have shown that it is possible to select a few which may be taken as representative of the generalized profile. Specifically, Heberling et al. [8] and Weston et al. [2] have suggested a Systems Connectivity Model (SCM) to assess the role of alterations in the relative abundance of *Clostridium, Desulfovibrio* and *Bifidobacterium* genera within the gut in the pathogenesis of ASD. This was carried forward in Ref. [2] by the use of an Agent-Based Model (ABM) which was employed to produce a unique simulation capable of examining gut-related risk factors for the development of autism. The ABM demonstrated that the introduction and tracking of changes in the relative proportions of these species in the gut from initialization to steady-state could be used to model the contribution of changes in the gut flora to an abnormal metabolome, which in turn is potentially related to the development of ASD.

This motivated the present attempt to take the process a step further by exploring the feasibility of using the Cellular Automaton (CA) model to test this type of hypothesis in multiple reiterations. The purpose is to provide a model for use in cases where experimental testing would involve too much effort, time and expense, or be overly invasive. If successful, this would demonstrate that CA modeling is capable of being used as a development and testing tool in such situations. In this paper, we aim to create a mathematical CA model which can measure the contribution of an altered gut microbiome to the risk of developing ASD. The assumption by Weston et al. [2] that three bacterial species, viz. *Bifidobacterium, Clostridium* and *Desulfovibrio*, are adequate to model the autistic gut at a minimally but usefully representative level has been adapted for use in the present CA model.

The scope of the use of the CA model in this paper is insufficient to allow its efficacy as a predictive tool in the medical treatment of ASD patients, and is designed only to demonstrate its potential as a cost-effective modeling tool in the study of ASD and the gut microbiome.

In 1966, Von Neumann advanced the idea of CA modeling as a type of dynamic system modeling tool which utilized three separately defined limited variables in the fields of time, space and state [50,51]. This has been used in many diverse fields of research, including work on an artificial brain [52], complex urban development [53], and simulation of important diseases. In Ref. [53], the authors have used it to simulate bacterial gene information sharing. Approaches to modeling biological systems using CA are described in Ref. [54]. The mechanisms of bacterial growth and division are described in Ref. [54] using the organism *Escherichia coli (E. coli)*. In Ref. [55] a theoretical CA-based model has been used to simulate the spread of bacterial agents during an epidemic.

All these studies have shown the successful use of CA modeling in the simulation of biological systems.

The current paper is organized as follows: Section 1 provides an introduction to the subject of investigation with some notes on its practical importance to research on the successful therapy of ASD. Section 2 describes the methodology, and provides the background of CA with respect to our model, including the set of CA rules and an algorithm for simulation. The results obtained using these techniques are described in Section 3. Section 4 is the discussion on the implication of these results. Finally, the conclusions drawn are presented in Section 5.

2. Methods

2.1. Definitions

The formal definition is given below:
Let

- CA_{space} be a finite grid (the elements of CA_{space} are called Cells),
- CA_{state} be a finite number of states,
- $CA_{neighbor}$ be a finite set (of size n = $CA_{neighbor}$) of neighborhood indices such that $\forall\ a \in CA_{neighbor}$, $\forall\ b \in CA_{space}$: $a + b \in CA_{space}$,
- $CA_{transitionrule} : CA_{state}^{n} \rightarrow CA_{state}$ be a transition function.

Then the 4-tuple (CA_{space}, CA_{state}, $CA_{neighbor}$, $CA_{transitionrule}$) is called a cellular automaton. John Conway's Game of Life [56] is a cellular automaton in which rules are defined for an infinite 2-*D orthogonal grid of* cells as shown in Fig. 1.

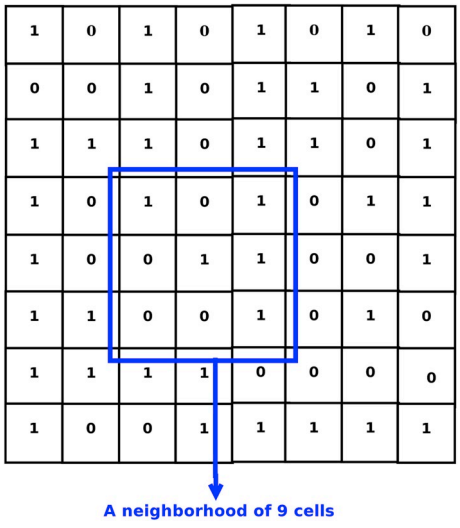


Fig. 1. Two Dimensional Cellular Automata: each cell contains a value either 0 (dead) or 1 (live); a box within the area outlined in blue represents one which is within the neighborhood of 9 cells (Moore neighborhood); the center cell in the nine-cell neighborhood cell represents $Cell_{i,j}$.

2.2. Rules

The set of rules is given below:

- If a cell state is 0 at time t and if exactly three of the eight neighbouring cells are in state 1, then the cell state becomes 1 at time $t + 1$.
- If a cell state is 1 at time t and if none, one or more than three of its neighbors are in state 1, then cell state becomes 0 at time $t + 1$.
- If none of the above two cases are true it remains in the same cell state.

Let us assume i and j are the index of the grid at time t, Then the transition rule is expressed as:

$$Cell_{i,j}^{t+1} = \begin{cases} 0, & \text{if } (\sum_{m=-1,n=-1}^{m=1.n=1} Cell_{i+m,j+n})) - Cell_{i,j} \leq 1 \\ 0, & \text{if } (\sum_{m=-1,n=-1}^{m=1.n=1} Cell_{i+m,j+n})) - Cell_{i,j} \geq 4 \\ 1, & \text{if } (\sum_{m=-1,n=-1}^{m=1.n=1} Cell_{i+m,j+n})) - Cell_{i,j} = 3 \\ Cell_{i,j}^{t}, & \text{otherwise} \end{cases} \quad (1)$$

Let us consider the *Moore* neighborhood of a cell $Cell_{i,j}$, where the neighbors are $Cell_{i+1,j-1}$, $Cell_{i+1,j}$, $Cell_{i+1,j+1}$, $Cell_{i,j-1}$, $Cell_{i,j+1}$, $Cell_{i-1,j-1}$, $Cell_{i-1,j}$, $Cell_{i-1,j+1}$.

The Game of Life was the motivation to apply the CA model to the gut microbiome, to demonstrate its utility in assessing the contribution of gut factors to predicting the level of risk for the development of ASD. The main objective of this work is to demonstrate the use of CA in predicting a change in the risk of ASD resulting from changes in the gut bacterial population. We developed a 2-D CA model to represent each of the three selected bacterial populations, namely, *Bifidobacterium (B), Clostridium (C)* and *Desulfovibrio (D)*, along with its health factor and the preferred food substrate. The proposed model yields the safe state when high levels of B and low levels of C and D are attained. When there is a high level of C with a low level of B, the unsafe state results. The model also includes the addition of lysozyme at time $t = k$.

The next section describes the proposed Cellular Automaton ASD-Gut Model (CA-ASDGM) set up under the defined rules and algorithms to simulate ASD risk based on the changes in the selected species.

2.3. Cellular automaton ASD-Gut Model (CA-ASDGM)

CA-ASDGM is designed as a set of CA rules to determine the contribution of gut bacteria to the risk of development of ASD. Based on these rules we have proposed an algorithm that can be used as a tool to predict the possibility of occurrence of ASD.

In the current research, all data on the microbial composition of the gut is based on reported bacterial populations derived from fecal microbial analysis using pyrosequencing of DNA as well as culture of live bacterial strains [15]. All the parameters used in our CA-ASDGM are represented in Table 1.

Our aim is to model population shifts occurring dynamically in three bacterial species whose relative proportions vary broadly between children with ASD and neurotypical children, namely, *Bifidobacterium, Clostridium*, and *Desulfovibrio*. Each of these species responds to the availability of different nutrients in the gut. Specifically, *Bifidobacterium* responds to glucose, lactose and fructo-oligosaccharides while their growth is inhibited by lysozymes as well as by an increase in the number of *Desulfovibrio*.

Desulfovibrio responds to chondroitin sulfate and lactate which is produced by *Bifidobacterium*.

Clostridium difficile uses low-molecular weight peptides preferentially, and glutamate dehydrogenase is important in its utilization of glutamate as a source of energy. However, most *Clostridium* species can also use the same compounds as *Bifidobacterium*, and are inhibited by *lysozymes* as well as by an increase in the population of *Bifidobacterium*.

Lysozymes are cellular enzymes that inhibit *Clostridium* growth, thereby inducing steady-state *Clostridium* populations at a lower level than before.

These three are used as archetypal species, since most other species may be assumed to act similarly to these. Thus these act as lumped variables, accounting for the simplicity of the present model [34].

In the CA-ASDGM each bacterial species is placed randomly on *Xmax* (50) and *Ymax* (50), with its preferred food substrates as mentioned in Ref. [2], within the grid cells of the 2-D CA model.

Each grid cell is defined by three parameters:

1. Type
2. Food
3. Health

The bacteria interact with the environment and reproduce, and the resulting rates of bacterial proliferation for each of these species are used, to generate a score according to the set of rules described below. These rules reflect two specific outcomes: growth and death. At initialization (time step $t = 0$) we plotted a low level of bacteria B, C and D. The starting availability of each preferred nutrient is plotted in every cell along with the health score, which is assigned as described below.

Each bacterium responds to the availability of its preferred substrate, as mentioned in Ref. [2], by varying rates of proliferation, and is assigned a bacterial health score. At each time step t from 0 to MaxTime, all bacteria were plotted with respect to their consumption of available nutrients and increase in the health score. Once the health score reaches the threshold level (DThreshold), cell division occurs leading to the creation of two daughter cells, increasing the bacterial population as shown in Algorithm 3.

At each time step the health score of the bacterium is reduced by the cost of living. Bacterial death may occur in either of two situations: if the health score is reduced below the threshold for the cost of living (IThreshold), or if the nutrient supply from the surrounding cells becomes nil. Our model uses the observed dynamic shifts in bacterial

Table 1
Parameters used in CA-ASDG model.

Variable name	Values	Description
X_{max} and Y_{max}	50 by 50	Size of the grid with Xmax rows and Ymax columns
Health	0 to 20	Initially 0 (Health Score of first bacteria deposited randomly on grid)
Food.type	I,G,F,L,T,C	Inulin (L), Glucose (G), Lactose (L), fructo-oligosaccharides (F), Lactate (T), Chondroitin sulfate (C)
Food.val	5	Score of corresponding food (nutrient/prebiotic/probiotic) first deposited on grid
Type	B,C and D	The type of bacteria placed on the grid, *Bifidobacterium* as *B*, *Clostridium* as *C*, and *Desulfovibrio* as *D*
DThreshold	50	Threshold of health score above which cell division occurs
IThreshold	5	Threshold of health score at which cell death occurs
cost_of_living	2	Health cost for each iteration
Δ	1	Amount of food consumed by the bacterium from its surrounding cells
replenish	1	Amount of food that is replenished at each iteration
lyso_time	t = k	Point of time of introduction of lysozyme

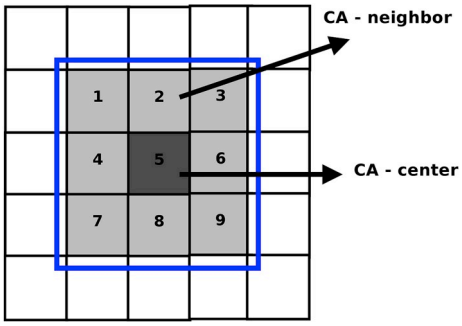


Fig. 2. Geometry of CA-center and CA-neighbor; numeral 5 represents the CA-center and remaining cells are CS-neighbors.

populations to determine the evolution of a safe or unsafe state which in turn predicts the risk of ASD in infants, as shown in Fig. 6.

On the basis of the *CA* model defined in the 2.1, *CA-ASDGM* has four parts which includes CA_{space}, CA_{state}, $CA_{neighborhood}$ and $CA_{transitionrule}$.

- CA_{space}: A 2-D Cellular Automaton is used with a neighborhood of nine cells (Moore neighborhood); the center cell in the 9 neighborhood cell represents CA-center as $Cell_{i,j}$ and its neighbors are as shown in Fig. 2.
- CA_{state}: Each Cell in a CA_{space} contains a group of three parameters *Type*, *Food*, and *Health*. The parameters *Food* and *Health* are represented by positive integers, and a character is used for the parameter *Type* as shown in Fig. 3.

$Cell_{ij} = [Type_{ij}, Food_{ij}, Health_{ij}]$

Where

- $Type_{ij}$ represents the bacterial type, namely *Bifidobacterium* as *B*, *Clostridium* as *C* and *Desulfovibrio* as *D*.
- $Food_{ij}$ represents *food*. *type* and *food*. *val* where *food*. *type* is the type of food substrate supplied such as nutrient, prebiotic or probiotic and *food*. *val* is the amount of nutrients in a cell with the values 0 to $Food_{Max}$, where 0 means no availability of nutrients, and $Food_{Max}$ means maximum nutrients.
- $Health_{ij}$ represents the bacterial health score with values 0,1,2,3, ..., upto $Health_{Max}$, where 0 means cell death, and $Health_{Max}$ means sound health score.
  - $CA_{neighborhood}$: A 9-neighborhood cell is considered a Moore neighborhood.
  - $CA_{transitionrules}$: The set of rules are given as follows:
- A cell's health is updated only when it obtains the required type of

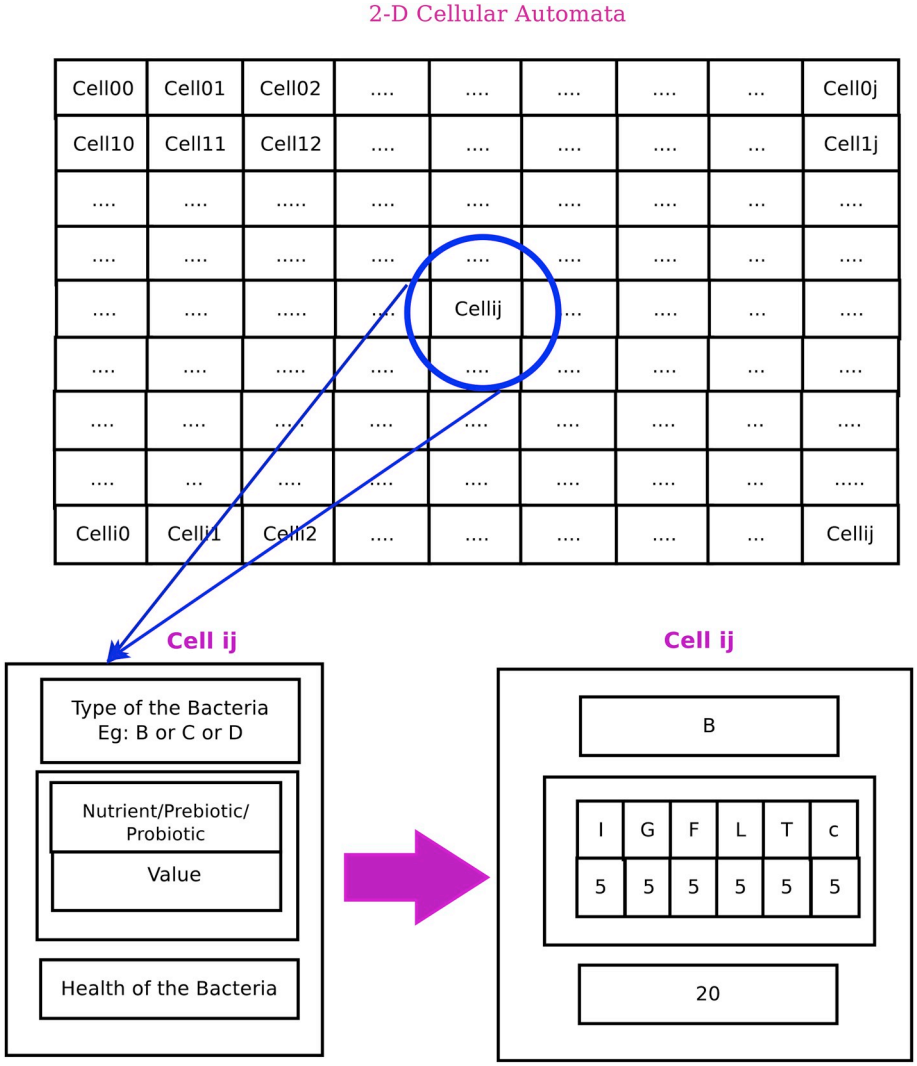


Fig. 3. A 2-D Cellular Automaton grid, each cell representing (1) Type of bacterium (Bifidobacterium (B), Clostridium (C), Desulfovibrio (D)), (2) Preferred food substrate or prebiotic or probiotic (Inulin (L), Glucose (G), Lactose (L), fructo-oligosaccharides (F), Lactate (T), Chondroitin sulfate (C)), (3) Health score of the bacterial cell.

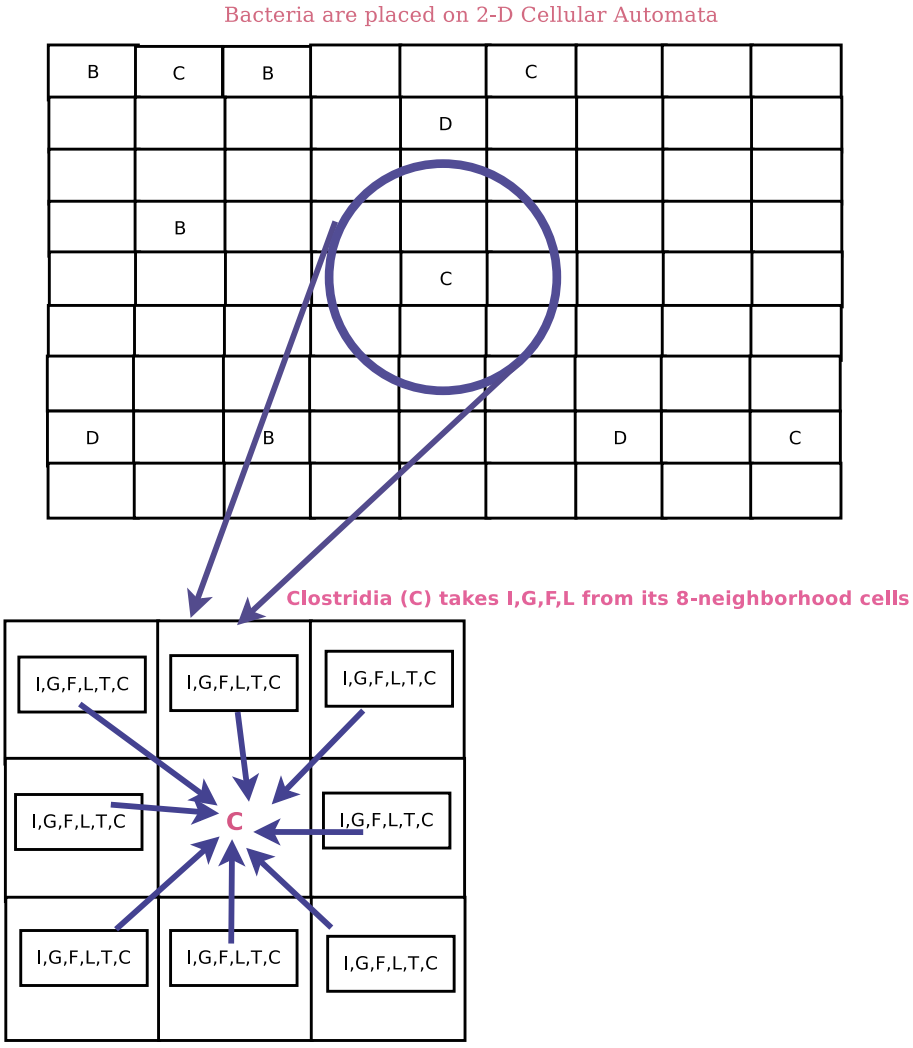


Fig. 4. The bacterium on any current grid cell can consume available nutrients from its eight neighbor cells to improve its health score.

nutrients from its $CA_{neighborhood}$. This results in the reduction of nutrients in the $CA_{neighborhood}$ cell.

– When the health score of a particular bacterium, say C, is updated, the value of food in the neighborhood cells will be updated as

$$food\_value^{new}_{neighborhoodcell} = food\_value^{old}_{neighborhoodcell} - \Delta \tag{2}$$

While the health score of bacterium C is updated as

$$C^{new}_{health\_score} = C^{old}_{health\_score} + \sum_{\forall\, neighborhood} \Delta \tag{3}$$

where $\Delta = food\_value$ given to C.

– $Health_{ij}$ updates the health score of the particular type of bacterium at each grid cell at each time step t based on its consumption of available nutrient from its neighboring cells.

Now each grid cell can be classified as any one of the following: i. Feeding, ii. Division, iii. Death, iv. Adjustment, and v. Growth.

i. Feeding: The bacteria on the current grid cell can consume available required nutrients from its $CA_{neighborhood}$ cells as shown in Fig. 4. This results in the reduction of nutrients from each of the $CA_{neighborhood}$ cells, while the health score of the current grid cell improves by an amount equivalent to the sum of the food values given by each of the $CA_{neighborhood}$ cells. This approach is utilized in developing Algorithm 2.

ii. Division: Once the current grid cell's health score reaches its threshold value, it can create two new daughter cells which are placed in any available empty cells in its neighborhood as shown in Fig. 5. The current grid cell's health score is shared between the two new cells. This becomes the value of the health score of each of the new daughter cells, namely, half of the current grid cell's health score multiplied by a probability p [57]. This is termed as division of the current grid cell. The current grid cell's heath score becomes zero. This concept of division is the basis of Algorithm 3.

iii. Death: As the health score of the current grid cell is reduced at each time step, the cell may be destroyed eventually in either of the following two cases, viz., (i) as soon as the health score drops below the threshold value, (ii) if no nutrients are available for consumption in the neighborhood cells.

iv. Adjustment: The introduction of *lysozyme* as nutrient results in a large reduction in the bacterial health score of Type C, decreasing its energy, as shown in Algorithm 4.

v. Growth: At each time step, the amount of nutrients in each cell is replenished.

Algorithm 1 is designed according to the set of rules for the CA-ASDGM described in Section 2.2. We have used the same quantitative measure proposed in [2], namely, the Gut Bacterial Index (GBI),

$$\mathrm{GBI} = \frac{[\mathrm{B}/\mathrm{B_s}]}{[(\mathrm{C}+\mathrm{D})/(\mathrm{C_s}+\mathrm{D_s})]} - 1 \tag{4}$$

Where Bs, Cs, Ds represents the average steady-state value for B,C, and D respectively.

Algorithm 1. Cellular Automaton to Find the Risk of ASD.

Algorithm 1 Cellular Automaton to Find the Risk of ASD

$X_{max} \leftarrow Row\_Grid\_Size$; $Y_{max} \leftarrow Column\_Grid\_Size$
$X \leftarrow 0 : X_{max}$; $Y \leftarrow 0 : Y_{max}$
$DThreshold \leftarrow 50$; $IThreshold \leftarrow 5$
$t \leftarrow 0$
$lyso\_time \leftarrow time\_step\_lysozyme$
$Cell[X][Y] \leftarrow \{type, food, health\}$ /* 1. *type* represents type of the bacteria (B-*Bifidobacterium*, C-*Clostridium*, D-*Desulfovibrio*), 2. *food* represents nutrient/prebiotic/probiotic (I/G/F/L/T/C), 3. *health* represents health of the bacteria. All the parameters are shown in Table 1 */
Initialize grid randomly $Cell[X][Y]$ with bacteria B, C, D and nutrients or prebiotic, probiotics and corresponding bacteria health score.
while $t < timesteps$ **do**
  for X:=0 to X_{max} **do**
    for Y:=0 to Y_{max} **do**
      if $Cell[X][Y].health \neq 0$ **then**
        Check which bacterium can feed on available nutrient
        Increase the health score of particular bacteria.
        Call Algorithm 2.
      if $Cell[X][Y].health > DThreshold$ **then**
        Division of the cell occurs
        Call Algorithm 3.
        $Cell[X][Y].health$:=0
      if $Cell[X][Y].health > IThreshold$ **then**
        Cell[X][Y].health-=IThreshold.
      else
        Cell may be destroyed eventually
        Cell[X][Y].health=0.
      if $t > lyso\_time$ **then**
        Time step when introducing lysozyme
        Call Algorithm 4.
  Count the number of each bacterial type B, C, D.
  $GBI = (B/B_s)/((C+D)/(C_s+D_s)) - 1$.
return

Algorithm 2. Consumption of Available Nutrients from Neighbor Cells.

Algorithm 2 Consumption of Available Nutrients from Neighbor Cells

$X \leftarrow XCurrentCellIndex$
$Y \leftarrow YCurrentCellIndex$
$r \leftarrow 0$
$\Delta \leftarrow 1$
for i:X-1 **do**X+1
  for j:=Y-1 **do**Y+1
    if $Cell[i][j].type == bacteriatype$ **then**
      if $Cell[i][j].food.type == nutrients/prebiotic/probiotic$ **then**
        if $Cell[i][j].food.val \neq 0$ **then**
          $Cell[i][j].food.val = Cell[i][j].food.val - \Delta$
          $r+ = \Delta$
    $Cell[X][Y].health = r$

Algorithm 3. Division of Cell.

Algorithm 3 Division of Cell

$x \leftarrow XCurrentCellIndex$
$y \leftarrow YCurrentCellIndex$
$p \leftarrow probabiltyparameter$
$Original\_cell\_health \leftarrow Cell[x][y].health/2$
$New\_D\_Cell\_Health \leftarrow original\_cell\_health * p$
$typeb \leftarrow Cell[x][y].type$
$fin \leftarrow 0$
$counter \leftarrow 0$
$xs \leftarrow \{x-1, x, x+1, x, x-1, x-1, x+1, x+1\}$
$ys \leftarrow \{y, y+1, y, y-1, y-1, y+1, y+1, y-1\}$
$c, d, index \leftarrow 0$
while $fin < 2$ and $counter < 8$ **do**
  $c = xs[index]$
  $d = ys[index]$
  if ($c >= 0$ and $c < X_{max}$ OR $d >= 0$ and $d < Y_{max}$) **then**
    if ($Cell[c][d].health := 0$) **then**
      $Cell[c][d].health = Original\_cell\_health$
      $Cell[c][d].type = typeb$
      $fin = 1$
  if $index < 7$ **then**
    $index := index + 1$
  else
    $index = 0$
  $counter := counter + 1$

Algorithm 4. Adjust when lysozyme added.

Algorithm 4 Adjust when lysozyme added

$X \leftarrow XCurrentCellIndex$
$Y \leftarrow YCurrentCellIndex$
$\Delta \leftarrow 2$
$typeb \leftarrow Cell[X][Y].type$
if $Cell[X][Y].health > IThreshold$ and $Cell[X][Y].type := C$ **then**
  $Cell[X][Y].health- = \Delta$

Where B_s, C_s, D_s represents the average steady-state value for *B*,*C*, and *D* respectively.

Lemma 2.1. *A system is in the safe state only if there exists a balanced bacterial count in the gut. This is represented by the calculated balanced factor GBI which is defined as in Equation* 4.

- *If the system is in the safe state, i.e balanced factor GBI* ≥ 0*, the risk for ASD development is low.*
- *If the system is in the unsafe state, i.e balanced factor GBI* < 0*, the risk for ASD development is increased.*

Based on Lemma 2.1 our model analyzes the risk level for the development of ASD.

3. Results

Our computational simulation results are based on three bacterial species, namely, *Bifidobacterium, Clostridium* and *Desulfovibrio*. Bacterial

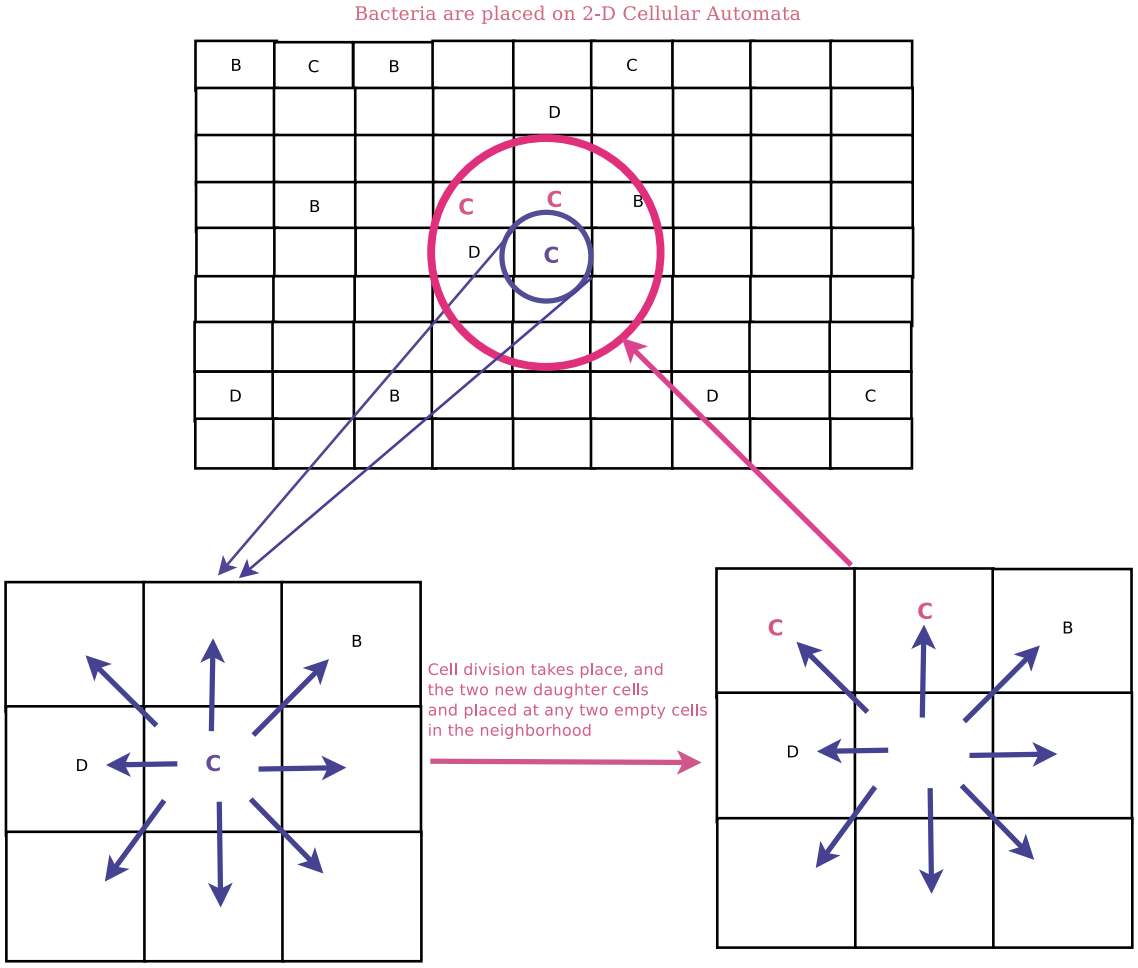


Fig. 5. The eight neighbor cells in which bacterial daughter cells can be placed. Once the health score of a bacterial cell falls below the threshold of health for cell division (blue), two new daughter cells are created (pink), which may be placed at any two empty cells in its neighborhood.

growth is dependent on the initial conditions supplied to the algorithm. In our model the initial levels of various bacterial species were altered at various time steps.

The CA-ASDGM showed a highly sensitive response to the variations in bacterial growth. Initially there was immediate and exponential bacterial growth, with subsequent rapid decreases in the growth rate, culminating in a steady state.

At the second level the population of *Clostridium* was increased, with the increase in growth being plotted at fixed time steps. This led to an initial observed dramatic reduction in the calculated balanced factor GBI, followed by oscillations between the safe and unsafe states. However, our model shows that the unsafe state predominates, indicating a higher risk for the development of ASD as shown in Fig. 7.

Conversely, a reduction in the concentration of *Clostridium* resulted in the return of the GBI to the safe state, indicating a lower level of risk for the development of ASD (Fig. 8). This finding suggests that *Clostridium* growth must be specifically targeted to optimize the gut microbiome composition such that a safe state is attained to minimize the risk of ASD development in infants.

Secondly, the simulation tested the possibility of minimizing *Clostridium* growth by increasing the levels of *Bifidobacterium* such that a safe state is attained, which in turn yields a very low probability of ASD development, as shown in Fig. 9. Such a reduction in *Clostridium* growth rate may also be achieved medically by treatment with the antibiotic vancomycin [2].

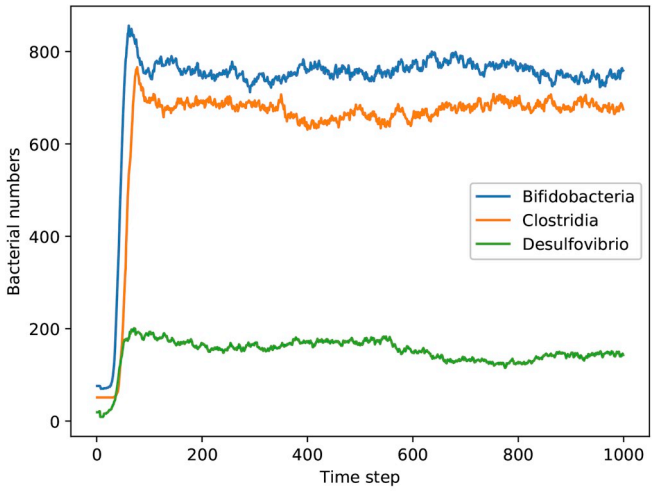
Thirdly, it is known that lysozyme, which is present in breast milk, targets Gram-positive bacteria such as *Clostridium* [58,59]. Based on this, the effect of adding *lysozyme* to the gut was simulated. A large effect was found when *lysozyme* was introduced into the gut [2], namely, a steep reduction in the *Clostridium* growth rate. This led to a rapid increase in the balanced factor GBI and the system entered the safe state, indicating a low risk for ASD. Hypothesis 3.2 satisfied Algorithm 1 and showed the safe state. Hypothesis 3.1 predicts the bacterial level of *Clostridium* at time $t = \infty$.

Hypothesis 3.1. *There is a predicted decrease in the bacterial growth rate of type Clostridium following lysozyme introduction at time $t = k$ resulting in a very low level of Clostridium at $t = \infty$.*

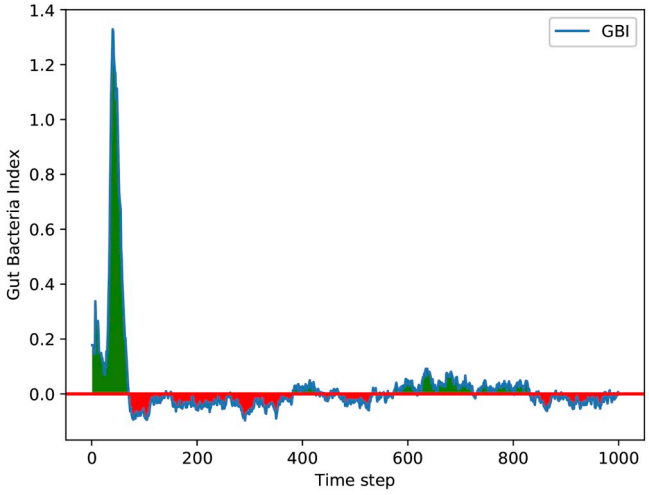
Let us assume that all bacterial types are initialized randomly. At each time step the bacterial growth rate depends upon the availability of nutrients in the neighbour cells. If nutrients are available, the bacteria consume nutrients from neighbouring cells and the health score goes up.

$$Cell^{t}_{i,j}.\ health = \begin{cases} \sum_{k=-1,l=-1}^{k=1.l=1} Cell_{i+k,j+l}.\ food) & \text{if } (\ \forall\ Cell_{i+k,j+l})) \\ 0, & \text{otherwise} \end{cases} \quad (5)$$

If $Cell^{t}_{i,j}.\ health \geq Dthreshold$ the cell divides and the daughter cells may be located in any of the empty neighboring spaces, resulting in an increase in the total bacterial count on the grid. At step $t = k$, the bacterial counts, namely *Bifidobacterium, Clostridium*, and *Disulfovibrio*, are represented by B_k, C_k and D_k respectively. At step $t = k + 1$, with the addition of *lysozyme* as nutrient, the health score of *Clostridium* is reduced. $Cell^{t}_{i,j}.\ health$ is decreased in relation to the incremental increase in the level of *lysozyme* at each time step. When *Clostridium* $Cell^{t}_{i,j}.\ health$ drops below the threshold level, the cell value becomes 0. That is, at $t = \infty$ it results in a very low count of *Clostridium*.

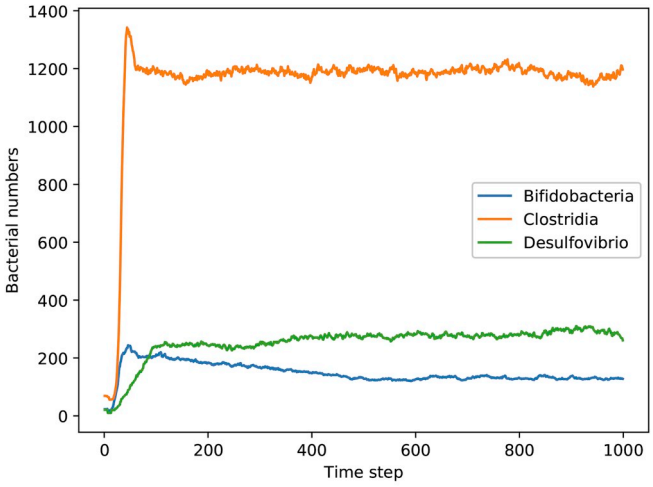


(a) Simulation of Gut Bacteria Growth

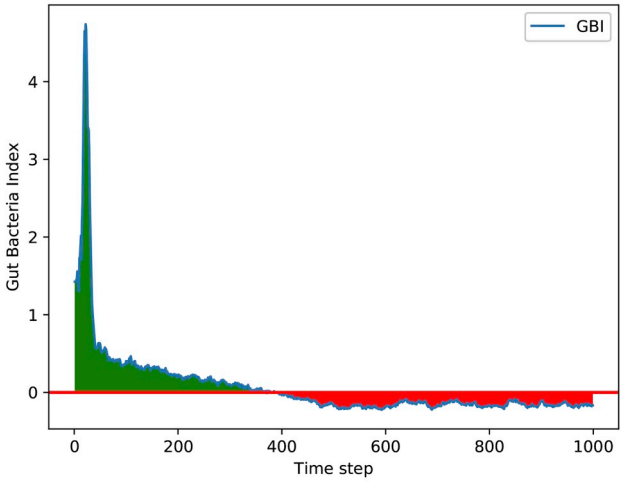


(b) Simulation of Gut Bacteria Index

Fig. 6. Gut Bacteria Development in normal healthy individuals: (6a) the initial bacteria supplied to the algorithm and observed bacterial growth in time steps.; (6b) the calculated balanced factor *GBI*; the red area indicates the risk for developing ASD.



(a) Simulation of Gut Bacteria Growth.



(b) Simulation of Gut Bacteria Index.

Fig. 7. Gut Bacteria Development when *Clostridium* growth rate is increased by 15%.

Hypothesis 3.2. *The risk of ASD is lowered following the addition of lysozyme.*

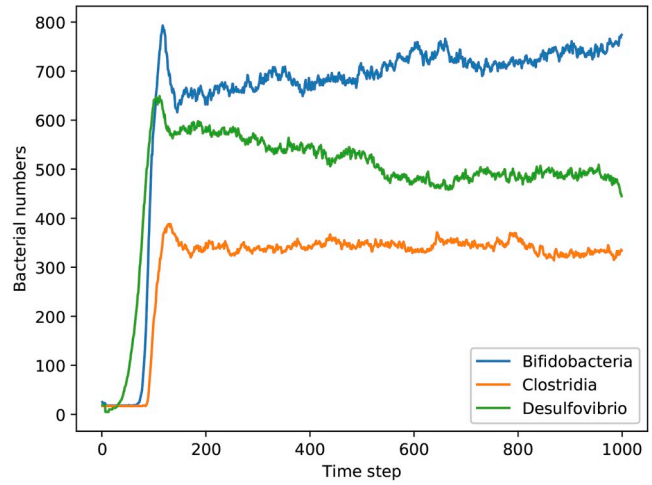
When the bacterial species *Bifidobacterium* (B), *Clostridium* (C), and *Disulfovibrio* (D) are assigned randomly, at time $t = 1$, the health score of the cell increases as it consumes the available nutrients in the neighbour cells. After reaching the health score threshold for division, the cell divides and the daughter cells occupy any of the empty neighboring cells. At $t = k$, the bacterial count may increase or decrease. The bacterial counts B_k, C_k, and D_k are used to compute the GBI according to Lemma 2.1. At $t = k + 1$, *lysozyme* is added to the system. At this point Hypothesis 3.1 predicts low counts of *Clostridium*, and when GBI is calculated, Lemma 2.1 shows that the system is in the safe state.

4. Discussion

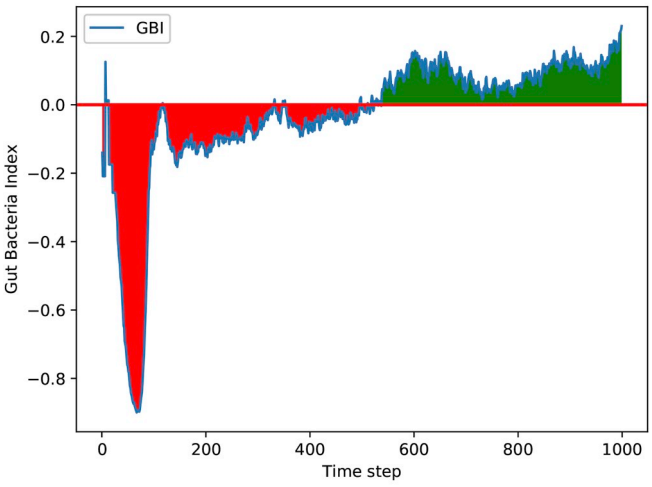
Many researchers have reported the role of environmental factors in the form of dietary alterations and changes in gut microbiota in the pathogenesis of ASD [46]. One study showed that *Firmicutes* were lower while *Bacteroidetes* were higher in children with ASD [24]. *Clostridium* species were described to be present in higher numbers in children with ASD. One possible mechanism of action was the production of metabolites such as phenols, indole derivatives and p-cresol which could be toxic to human cells. Other species which were reported at higher levels in ASD included the lactic acid bacilli *Enterococcus*, *Lactobacillus*, and *Streptococcus* [5,32], *Bacteroidetes* such as *Bacteroides, Odoribacter*, some *Prevotella* and *Alistipes* species, *Sutterella* and *Enterobacteriaceae*, and *Akkermansia*. Using indices of certain specific microbes like *Clostridium* and specific metabolite levels could potentially serve as a marker of ASD risk.

Gastrointestinal symptoms are reported to occur at higher frequencies in children with ASD though the type and intensity of the disturbances vary from 23% to 70% across studies [60,61]. They may also contribute to the severity of the condition [60], as they may produce both emotional and behavioral issues, and even more, because they are due to an imbalance in the gut microbiome that affects brain function [62–64].

In Ref. [65], some phenols have been shown to be raised in ASD, and are related to the activity of *Clostridium*, which is among the most abundant species in the gut microbiota in ASD. One possible mechanism of action was hypothesized to be the production of metabolites such as phenol and indole derivatives, free amino acids and VOCs which could be toxic to human cells or which are known to participate

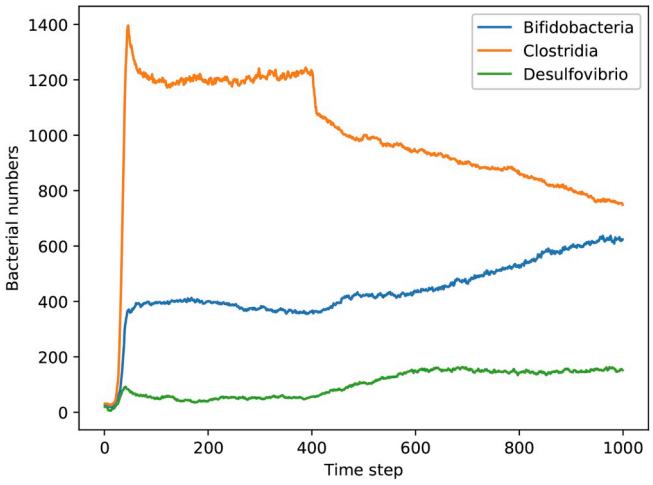


(a) Simulation of Gut Bacteria Growth

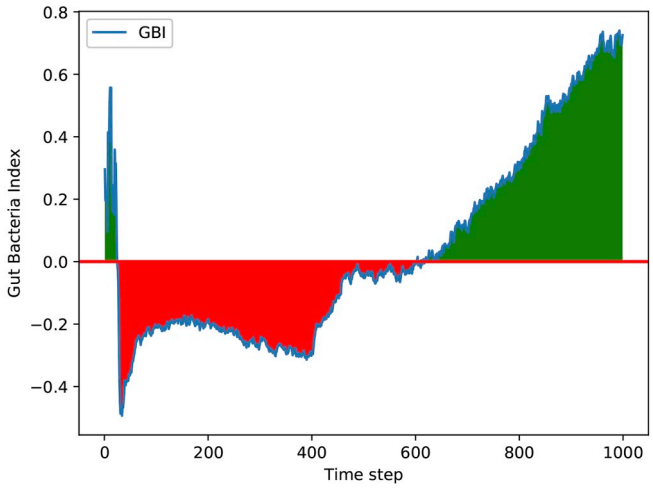


(b) Gut Bacteria Index

Fig. 8. Gut Bacteria Development when *Clostridium* growth rate is decreased by 15%.



(a) Simulation of Gut Bacteria Growth



(b) Gut Bacteria Index

Fig. 9. Gut Bacteria Development following *lysozyme* introduction: the effect of adding lysozyme to the gut. This leads to a rapid increase in the balanced factor GBI such that the system enters the safe state.

in the pathogenesis of certain neuropsychiatric disorders [24,29,30,34,35,37,41]. Exposure to abnormally high concentrations of these phenols is considered to contribute to the severe behavioural and cognitive impairments in ASD [37,39].

The observation that a high ASD risk or unsafe state results from an increase in the *Clostridium* population in our model agrees with the finding of several researchers that the *Bacteroidetes* and *Clostridium* genera are associated with the highest levels of free amino acids and volatile organic acids [66,67]. These studies are in agreement with our finding that curtailing the growth rate and population of certain specific microbes like *Clostridium* could potentially help to assess and mitigate ASD risk.

This also agrees with our finding that reducing the population of *Clostridium* leads to the emergence of a safe state with a lowered risk of ASD development. Overall, children with ASD have a less diverse gut microbiome with some genera being relatively less abundant [68]. It is important to understand that gut bacteria have complex interactions which allow the human diet to be highly varied while still being digested. Different methods of sampling, different sampling sizes and differences in the methods used to characterize the microbes, as well as the broad variability in the symptoms and signs of the disorder itself, may account for the striking variations in the conclusions of different investigators as to the type of organisms found to predominate in the gut of children with ASD. For instance, one paper describes *Prevotella, Coprococcus* and *Veillonellaceae* as being relatively less abundant in the gut of children with ASD. On the other hand, numerous studies have reported increased numbers of *Clostridium* [26,28,29]. Other confounding factors include variations in the diet, use of oral antibiotics, and use of prebiotics or probiotics.

For the purpose of the current study, which aimed to demonstrate the feasibility of the CA model in determining the risk for ASD, the number of species was reduced to three. The assumption is that changes in these species would mirror on a small scale the imbalances occurring in the gut microbiome of children with ASD that apparently act as risk factors in the development of ASD.

The 2-D CA-ASDGM approach is suitable to analyze the dynamic shifts in the relative numbers of representative bacterial species in the gut microbiome so as to successfully predict the risk of developing ASD. By selectively changing specific parameters in the current study, the model simulates physiological shifts that could cause the development of a high-risk bacterial environment for ASD. Moreover, it is able to demonstrate the potential efficacy of adding *lysozyme* to the nutrient feed to target the *Clostridial* population and bring the system to a safe state. This is in good agreement with the observed reduction in *Clostridium* following lysozyme-rich milk feeds [59].

The CA-ASDGM model may therefore be considered a feasible testing tool for multiple hypotheses dealing with shifts in various parameters affecting specific genera in the gut microbiome, and their effect on the risk of ASD. The importance of this approach is not limited to ASD, since it may be extended to other conditions in which the gut microbiome may contribute to disease pathogenesis and severity, such as obesity and inflammatory bowel disease [59]. The ability to modulate various parameters and to predict dynamic changes in bacterial populations in correlation with the progress of the disease means that its use could be further extended to study individual patients.

5. Conclusion

Our Cellular Automaton Autism Spectrum Disorder Gut Model (CA-ASDGM) is a mathematical model in which three bacterial types (*Bifidobacterium, Clostridium*, and *Desulfovibrio*) are used to represent, on a minimal scale, the key bacteria in the gut microbiome that are involved in the risk of developing autism.

We constructed a 2-D Cellular Automaton. The three bacteria are positioned randomly in the cells along with the health score and food score of each. The populations of these three bacteria were altered in our model using the Cellular Automaton approach. Each bacterial population was observed to undergo growth and death in proportion to its health factor and preferred nutrient availability. Low initial bacterial levels were simulated in order to identify the particular changes that resulted in a safe state. Following an increase in the population of *Clostridia*, the model showed the emergence of an unsafe state of the gut microbiome, which is associated with a higher risk for the development of autism. This model therefore suggests that *Clostridium* growth in the infant gut should be targeted to reduce the risk of developing ASD.

The model also explored the effect of introducing *lysozyme* into the gut as nutrient, which resulted in a safe state by producing a reduction in *Clostridial* growth. It is important to remember, however, that currently, it is still not clear what degree and nature of risk is imparted by various and diverse risk factors to the development of this spectrum of disorders. This study is intended to add to the sum of knowledge by contributing a modeling tool with the potential to simulate various changes in the gut microbiome as represented by three archetypal bacterial species, and their impact on the risk of development of this condition. Further studies are required to test this hypothesis and elucidate the relationship between autism and the gut.

Conflicts of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compbiomed.2019.05.015.

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