Using multi-level Petri nets models to simulate microbiota resistance to antibiotics

Using Multi-level Petri Nets Models to Simulate Microbiota Resistance to Antibiotics

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***Abstract*—The spread of antibiotic resistance is a growing problem known to be caused by antibiotic usage itself. This problem can be analyzed at different levels. Antibiotic administration policies and practices affect the societal system, which is made by human individuals and by their relations. Individuals developing resistance interact with each other and with the environment while receiving antibiotic treatments moving the problem at a different level of analysis. Each individual can be further see as a meta-organism together with his associated microbiotas, which prove to have a prominent role in the resistance spreading dynamics. Eventually, in each microbiota, population dynamics and vertical or horizontal transfer events implement cellular and molecular mechanisms for resistance spreading and possibly for its prevention. Using the Nets-within-nets formalism, in this work we model the relation between different antibiotic administration protocols and resistance spread dynamics both at the human population and at the single microbiota level.**

***Index Terms*—Antibiotic resistance, human microbiota, hybrid models, multilevel models, Petri Nets, Nets-within-nets.**

I. Introduction

Multicellular organisms are meta-organisms comprising both a macroscopic host and its symbiotic microbiota. The catalog of these organisms and their genes form the human microbiome [1]. These complex communities of microbes include: bacteria, fungi, viruses and other microbial and eukaryotic species. They provide a tremendous enzymatic capability and play a fundamental role in controlling several aspects of the host's physiology.

In the frame of this symbiotic relationship, bacteria and other organisms composing the microbiota normally do not express virulent traits. Indeed, one of their functions is to protect the host from pathogens [2]. The different species that form a microbiota populate their relative biological niche according to the respective growth rates, and to the overall availability of resources [3]. The more a species prevails, the more its genetic set does.

In this paper, we propose a computational tool to investigate how different antibiotic protocols can affect the spread of antibiotic resistance. The central question we address is the relation between antibiotic use and the spread of resistance, a problem we choose to approach at different levels: from health care management of antibiotic treatment policies and practices to individual patients as meta-organisms.

Healthy microbiotas share two common features: (i) a uniform core of functionalities in the corresponding microbiomes, and (ii) a great diversity in terms of species composition. This provides on the one side a set of guaranteed core functionalities and, on the other side, a great capability for plasticity [1]. The microbiota can recruit new functionalities in two ways. First, new species can join the population bringing their different functional sets that enlarge the functions of the established microbiota [4]. Second, horizontal transfer (HGT) mechanisms allow cells to acquire new functional capabilities [5]. The most studied HGT mechanism with respect to antibiotic resistance spread is the exchange of plasmids between bacterial cells of same or different species [6].

Since antibiotics target very conserved features [7], most times they hit a large spectrum of different bacterial species at once. After antibiotic administration, many bacterial cells (besides the pathogenic ones the attack was directed onto) perish, with the exception of those expressing antibiotic resistance for that molecule. After that, resistant bacteria have way less competition for resources, and gain advantage in colonizing the niche [8].

Exchanges of genetic information between bacterial cells take place through the external environment as well. Antibiotic resistance affects individual hosts and the system they live in. This may represent both an imminent risk for the society at the production systems level, and a threat for the environment in general [9].

Our model considers three main system levels. The top level refers to the population of human hosts, each represented by means of the specific microbiota it carries. At the middle level microbiotas are described in terms of the bacterial species they include. At the bottom level each species composing the microbiota is represented by means of a sample of each bacterial cells, either carrying resistance factors or not.

Our approach is based on Nets-Within-Nets (NWN), a high-level Petri Nets formalism supporting the development of multi-level and hybrid models suitable for stochastic and dynamic simulations [10]. Given their capability for exploring the system behaviour through simulation, predicting its evolution in time, and its variation in different conditions, these models can make good decision support tools for the development of innovative therapeutic protocols and for policy making in the health care context [11], [12].

II. Materials and methods

A. Nets-within-nets (NWN)

NWNs are a class of high-level Petri Nets supporting nested architectures where complex information attached to tokens can recursively be specified with the Petri Nets formalism [10]. NWNs provide encapsulation and selective communication. This suits well the modeling requirements posed by the considered problem, where different entities mostly evolve independently at different system levels and exchange information in a highly controlled and selective ways.

For model design and simulation we relied on Renew, an integrated tool supporting design and simulation of high-level Petri Nets [13].

B. Model construction

To study how antibiotic administration protocols affect insurgence spreading and severity of antibiotic resistance both within individual human microbiotas and across populations, we simulate resistance spreading in a control population not receiving any treatment. This population is compared with a population receiving antibiotics either under a traditional protocol or under a carefully designed administration protocol.

Figure 1 shows a high-level conceptual view of the proposed model organized into three hierarchical levels: (1) the human host (top level), (2) the microbiotas (middle level), and (3) the bacterial cells (the bottom level).

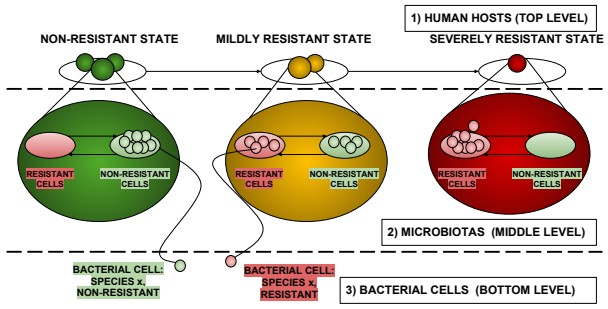

Figure 1. High level conceptual view of the proposed model.

Human hosts (top level): This level, whose complete model is reported in Figure 2, describes the population of human individuals in which antibiotics resistance can spread. As reported in Figure 2.A, this level is composed of three main places respectively modeling the possible health conditions for the microbiota: (i) absence of resistance factors reported in green, (ii) state of mild resistance reported in yellow, and (iii) state of severe resistance reported in red. Transitions between such places represent the progressive worsening of the antibiotic resistance state. Tokens at this level represent human individuals through their microbiotas. Each microbiota is a net whose structure is reported in Figure 3. At this level, each microbiota is associated with a *point prevalence score* (PPS). The PPS is dynamically computed as the proportion of resistant bacterial cells over the total bacterial population during the microbiota net simulation (Figure 3.A) [14]. The PPS is sampled from the upper level (Figure 2.D) to take decisions. When it exceeds a threshold, transitions move the microbiota token to the next stage of resistance progression (Figure 2.A). In this model, such effect is dictated primarily by the activation of antibiotic administration and microbiota re-integration events (Figure 2.B; Figure 3.B and Figure 3.F). These are supposed to affect the system both at the population level, where microbiotas exist as tokens (in Figure 2.C the net structure instantiating them is highlighted), and at the level of individual microbiotas (Figure 3). A back-end of custom Java classes tracks the PPS (Figure 3.D) as well as the numerosity of host individuals in the three different states (Figure 3.E).

Microbiotas (median level): individual microbiotas exist at the human hosts (top) level as instances of the general microbiota net depicted in Figure 3. In each instance, two places (Figure 3.C) represent the conditions bacterial cells (plain colored tokens) can fall into: (i) antibiotic resistance, depicted in red and (ii) non-resistance, depicted in green.

Resistant and non-resistant populations of bacterial cells in a microbiota engage into a competition based on the interplay between growth rates, the competition for limited resources, and HGT mechanisms able to transform non-resistant cells into resistant ones. The model represents each of these mechanisms by a specific structure in the net architecture. HGT are able to turn an existing non-resistant cell into a resistant one (Figure 3.G). Both resistant and non-resistant species contribute to the overall population numerosity, but through separate generative mechanism (Figure 3.D), which for simplicity in this model we associate with the same growth rates. Eventually, limited availability of resources bounds population growth (Figure 3.E). From the upper level (Figure 2) synchronous channels can provide inputs to the microbiota simulation: antibiotic administration events cause dose-dependent depletion of non-resistant cells (Figure 3.B), and microbiota re-integration causes a re-population of the state of non-resistance (Figure 3.F).

Bacterial cells (bottom level): tokens representing the bacterial cells composing the microbiota populate the places in Figure 3.C. These tokens are colored tokens that use the colors to carry information about species identity and the possible presence of resistance factors in the cell. They can be generated by either regular population growth (Figure 3.D) or possibly by re-integration of non-resistant cells (Figure 3.F).

Antibiotic administration and spread of resistance (cross-layer communication): both antibiotic administration and the subsequent progression towards a state of higher resistance involve more than one level in the model.

First, antibiotic administration origins at the human hosts (top) level (Figure 2.B). It affects all microbiota instances existing in the place it targets. Yet, the effects of such events affect each single involved microbiota (through the channel in Figure 3.B). They cause the depletion of all tokens representing non-resistant cells, dramatically affecting the overall target population dynamics.

Second, the passage from a state of resistance prevalence to the next one requires the enabling and activation of a transition between the corresponding places at the human hosts (top) level (Figure 2.A). This corresponds to the fulfillment of a specific requirement at the microbiota level. The PPS (computed in Figure 3.A) must cross the threshold to allow the corresponding upper level transition (Figure 2.A) to fire and thus to move the microbiota to the next resistance stage at the population level.

These two mechanisms involve readings of information originating from one level, and subsequent decision making processes based on another level. Such exchanges occur dynamically as the model evolves, engaging the different levels in a continuous crosstalk, connecting them through selected touch points. This requires suitable channels allowing for real-time selective communication. Renew supports the implementation

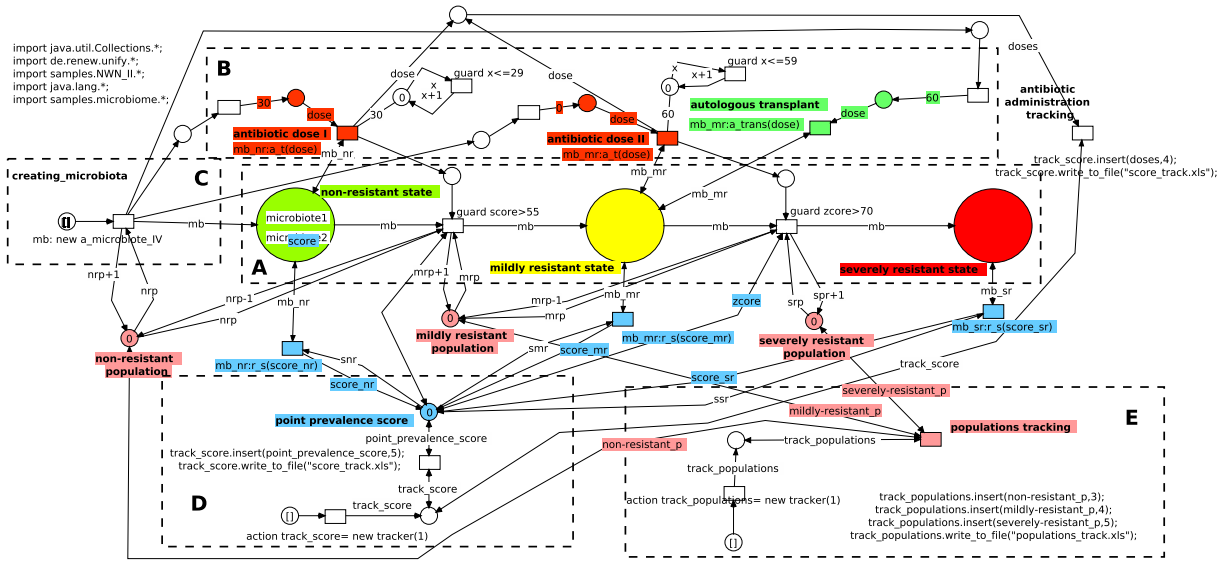

Figure 2. Net architecture for the human hosts (top) level. Three main places describe the health states the microbiotas can assume: non-resistant (in green), mildly resistant (in yellow) and severely resistant (in red) (A). Transitions can move microbiota tokens, each having the net structure from Figure 3, to the next place. The state of non-resistance holds two microbiota tokens depicted in a compact form, i.e., with their name only. This happens according to the value of their PPS. A synchronous channel (D) reads the PPS from nets at the lower level, possibly taking the relative microbiota to the next step along resistance progression. The structures in E track the changing numerosity of microbiota instances in each place). Synchronous channels take care of the antibiotic administration and microbiota integration events (B), activating net structures at the lower level (Figure 3.B and Figure 3.F, respectively) according to time delays and number of microbiota instances injected in the net by the dedicated structure (C).

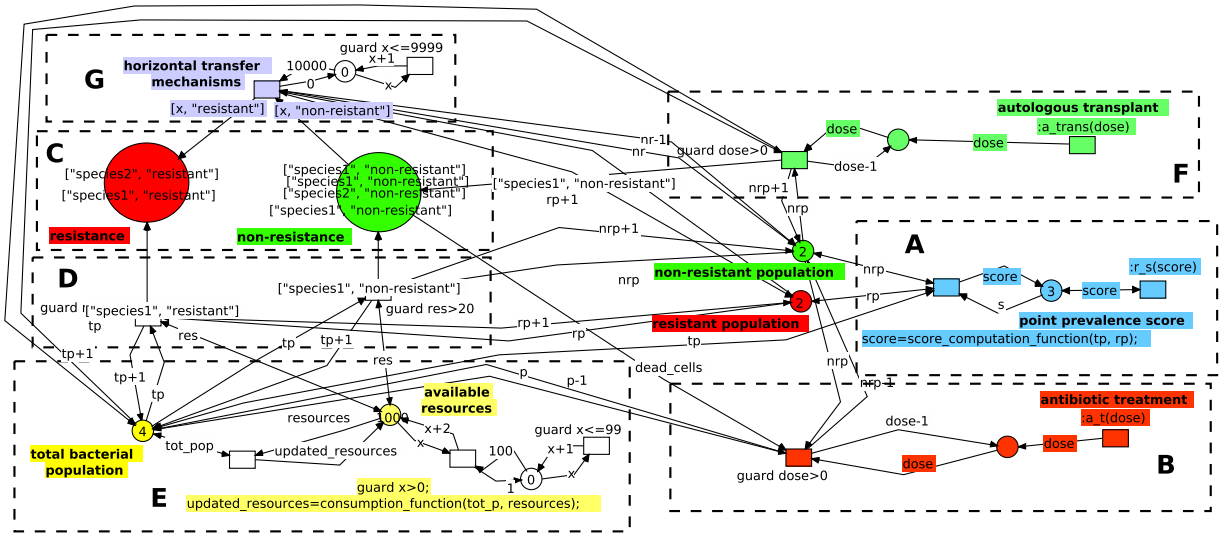

Figure 3. Net architecture for the microbiotas (median) level. Two main places describe two conditions each bacterial cell can assume: a state of non-resistance (green) and a state of resistance (red) (C). Horizontal transfer mechanisms can turn non-resistant cells into resistant ones (G). Net structures managing the generation of new bacterial cells (D), total population numerosity and resources availability (E) rise competitive population dynamics between resistant and non-resistant populations. Dose-dependent depletion of non-resistant cells following antibiotic administration (B) and microbiota reintegration (F) events activate as synchronous channels with structures in Figure 2.B. The structure in A dynamically computes the PPS of the microbiota net, making the information available for the upper level through a synchronous channel (see Figure 2.D)

of synchronous channels in NWN, thus fulfilling this precise requirement [13].

C. Tuning of the model

To perform simulations the model must be properly tuned with a careful aggregation of data from different sources.

In this work, we intend to present a proof of concept model. For this reason, we represent the large diversity of species composing a microbiota in an extremely simplified way. In fact, the scope of this study is not to study species diversity and how this is affected by antibiotic treatment (for an introduction to this problem, see [15]). Instead, this work aims at linking antibiotic administration protocols with the

spread of resistance in population of humans. This is done looking at the dynamics that describe the spread of resistance factors within individual microbiotas through horizontal transfer mechanisms, and given species-specific competitive population dynamics.

The proposed net architecture is based on a functional descriptions of the problem at the different levels [16], while species identity and antibiotic or resistance mechanisms are maintained at an abstract level. The ultimate goal is to provide a general instrument that can be easily adapted to the peculiarities of specific problems and use cases in the domain of interest.

III. Results and Discussion

To showcase the potential of the proposed multi-level and hybrid model, we designed two experiments studying the problem at different abstraction levels.

The first experiment aims at evaluating how standard antibiotic treatments can cause and speed-up the spread of resistance factors in a single microbiota (Figure 4). The second experiment is instead centered at the population level (Figure 5). Multiple instances of the microbiota net are generated at the top level to model a population of human hosts distributed into the three resistance states. Their dynamics is then simulated.

For both experiments, the initial setting of every simulation represents a state of health for the microbiota, where the non-resistant bacterial population largely prevails over the resistant one. This has been modeled with a ratio between non-resistant and resistant cells of 9:1, recapitulating semi-quantitative functional descriptions of a non-resistant microbiota.

In the first experiment (Figure 4), three different conditions are considered. Figure 4.A reports the case of absence of antibiotic treatment. Figure 4.B considers a traditional antibiotic administration protocol. Two administration events occur within a given time frame: the first provides a low dose, the second a high dose. Figure 4.C proposes an innovative treatment protocol. The administration scheme from the traditional protocol is maintained but, in parallel to the first low antibiotic dose, the microbiota is integrated with non-resistant cells for prevention purposes.

Simulations in this experiment aim at tracking the PPS of a single microbiota that reflects the presence of resistant cells within the total bacterial population. This metric is sampled at different significant stages of the simulation to capture the reaction of the system to the corresponding events. In particular, it is sampled: (1) before any treatment is administered; (2) after the administration of the first lower dose of antibiotic plus, for the innovative protocol, the microbiota integration, and (3) after the administration of the second higher dose of antibiotics.

The values presented are obtained averaging the PPS at each phase over 30 simulation runs. Given the observed variance across simulations, such number of runs guarantees a 5% error margin at a 95% confidence interval under all experimental designs. This type of simulation allows us to study the link between the overall resistance state of a microbiota and different kinds of treatments, given the internal population dynamics of resistant and non-resistant species.

When no treatment is provided, the PPS remains at a low and almost constant level along the simulation. Slight increases can be ascribed to the activation of horizontal gene transfer mechanisms or random mutations. Out of 30 experiments, only in 4 cases the score exceeded the threshold required to move the microbiota into a state of mild resistance. The relative averaged PPS (APPS) of 57.75 ± 0.63, reported in the second bar of Figure 4.D refers to these cases. All other cases are represented by the first bar of Figure 4.D, showing an APPS of 48.67 ± 0.61. In none of the cases the microbiota reached a state of severe resistance.

The traditional antibiotic treatment protocols, based on repeated administration events, are mimicked by the model in a simplistic way. Two administration events occur: the first provides a low dose, allowing for partial recovery of the non-resistant portion of the bacterial population; the second provides a high dose, taking the microbiota towards a state of severe resistance. In Figure 4.E, we observe the increase of the PPS, corresponding to what we consider the progressive spread of resistance within the microbiota. Before treatment (pre-treat), the APPS was 50.34 ± 0.88, as in the control condition. After the administration of the first dose (dose 1), a significantly APPS increase is observed compared to the control condition (APPS = 64.74 ± 1.14). After the second high-dosage administration event (dose 2), the resistance violently increases with a APPS of 93.24 ± 2.4, depicting what we consider as a state of severe resistance.

The innovative treatment protocol enriches the antibiotic administration procedure with a parallel preventive reintegration of non resistant bacterial cells. This enforces the non-resistant population and mitigates the advantage acquired by the resistant cells in the competition for colonizing the niche. This design is inspired by existing clinical practices such as autologous microbiota transplants, also called bacteriotherapy [4]. Such preventive action is performed in parallel with the administration of the first antibiotic dose. As shown in Figure 4.F, the pre-treatment APPS (pre-treat) is similar to the one of the previous experiments: 50.94 ± 0.67. With the addition of the preventive intervention (dose 1 + prev), significant differences can be seen already at the second stage. The APPS stops at 53.03 ± 0.52 showing a 18.08% decrease compared to the traditional treatment. Eventually, in the third stage (dose 2), the APPS reaches 70.75 ± 0.82 with a 24.12% decrease with respect to the traditional protocol.

Observing the sample tracks from the simulations, we can spot an example of a steady PPS when no treatment is administered (Figure 4.G). In Figure 4.H, in correspondence to the lower and higher peaks of the blue curve representing the antibiotic dosage administration time, the orange curve shows proportional increases. It then stabilizes on fixed values that correspond to the stages used to compute the APPS. The same dynamic can be observed in Figure 4.I, with the difference that the preventive action mitigates the effects of the first dose of antibiotics, taking the PPS to a level similar to pre-treatment. This takes time. In fact, right after the administration, a small spike is an indication of the effects the antibiotic would have otherwise.

In the second experiment we intend to center our observa-

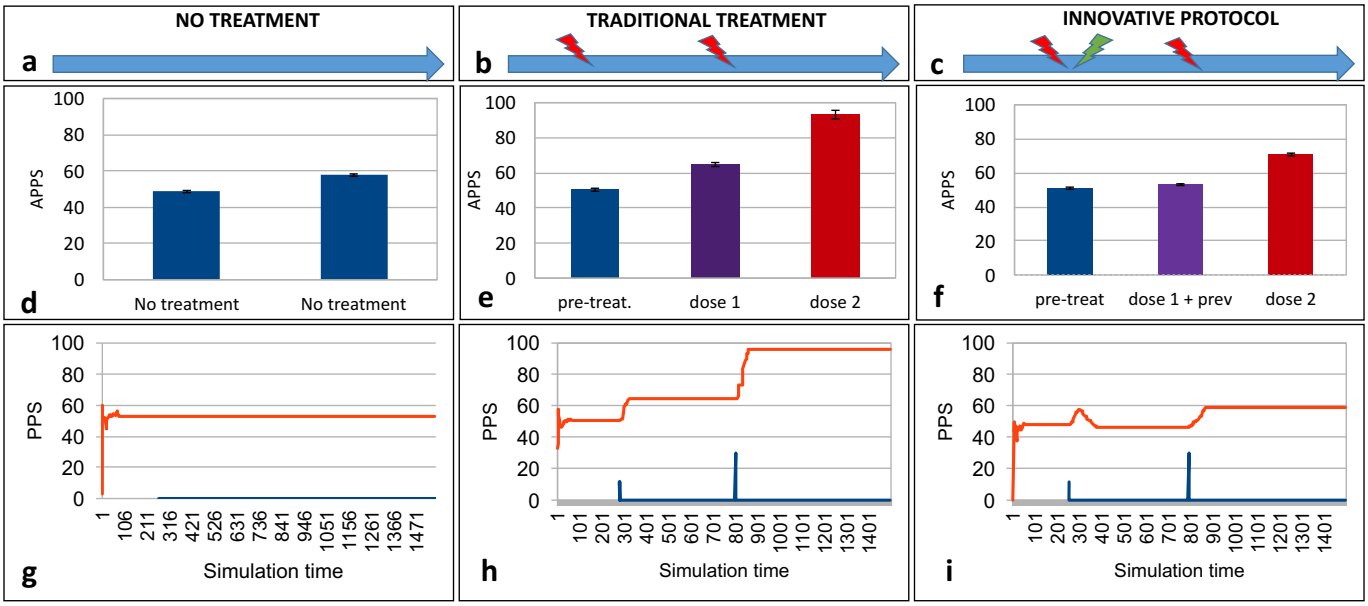

Figure 4. In the first experimental condition (a) no treatment administration occurs: the APPS is 48.67±0.61 (d), keeping the microbiota into the "non-resistant" state. Only in 4 cases such threshold was crossed, and the APPS for those cases is slightly higher: 57.75±0.63 (d). Most simulation tracks for the PPS (the curve in orange) reach a value and keep it steadily along simulation time (g). In the second experimental condition (b), two doses of antibiotic were administered to the microbiota, the first lower and the second higher. In (e) we report the APPS for the three significant stages of the experiment: before treatment, an APPS of 50.34±0.88 is homologous to that maintained in (d); after the first dose, APPS is 64.74±1.14, corresponding to a state of mild resistance; after the second higher dose APPS is 93.24±2.4, beyond the threshold for a state of severe resistance. The simulation track reported in (h) recapitulates the typical track for this case: after each dose (the curve in blue, whose peaks represent doses administration) PPS increases proportionally, moving from the non-resistant steady state, to the mildly resistant one, and finally to the severely resistant state, where it stays. In the third experimental condition (c) the same antibiotic administration of (b) is maintained, but in parallel to the first dose administration, a reintegration of the non-resistant bacterial population is performed. In (f) we observe how this lowers APPS both in the second and in the third stages of the experiment: while pretreatment APPS remains homologous to those in (d) and (e) (50.94±0.67), after the first dose of antibiotic and microbiota reintegration APPS drops to 53.03±0.52, and reaches after the second dose of antibiotics the value of 70.75±0.82, corresponding to significant decreases compared to the corresponding simulation stages in the traditional treatment scenario (e). In (i) we observe how the effects of the first antibiotic dose are counterbalanced by the microbiota reintegration: in a first moment APP begins to rise, but it is bounded right away to a low level by the preventive action, leaving on the track just a transient spike. After the second higher dosage of antibiotics, APP increases, reaching a steady state at a higher level, which is anyway lower that that reached in (h).

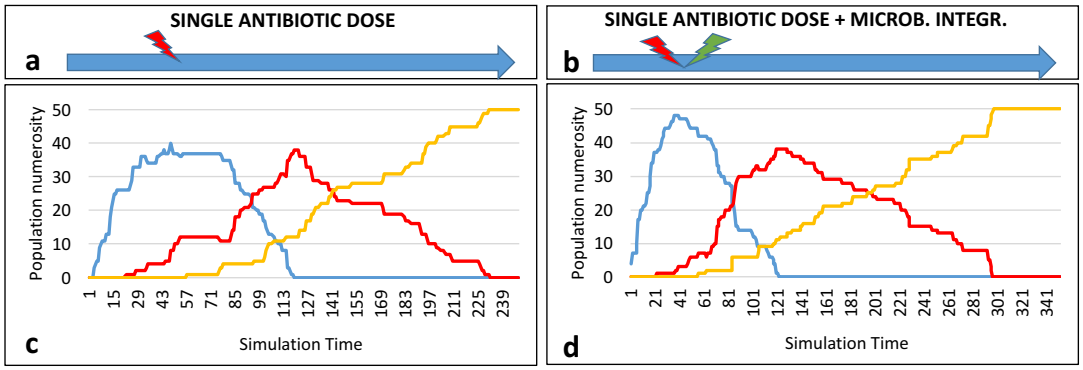

Figure 5. We tracked population numerosity inside each of the corresponding places at the top level in the model, representing states of non-resistance (the curve in blue), mild resistance (orange) and severe resistance (yellow), respectively. As a metric for how resistance can affect a population of hosts, we tracked the simulation time at which the state of severe resistance got more populated than the other two, describing it as the severe resistance onset time (T). Simulation time is indicated in terms of simulation cycles. In the first experimental setting (a), a single high dose of antibiotics was presented to each microbiota instance. This corresponded to an average T (AT) of 162.87±7.78. A typical track from these experiments is reported in (c), where healthy individuals (blue curve) progressively acquire resistance. Some of them reach severe resistance right away (yellow curve), while most of them pass through a phase of mild resistance (orange curve). In the second experimental condition (b), microbiota reintegration is performed in parallel to the single high antibiotic dose administration. This yields an higher AT (178.64±7.4) with respect to that observed with the antibiotics alone. In (d) we notice how in the typical track from this set of experiments the overall migration to healthy individuals towards a worsening resistant state slows down, resulting in higher values for T.

tions on the human hosts population level. Instead of creating a single instance of the microbiota net token, we created multiple instances in order to represent a population of human hosts.

In this experiment, we compare two experimental conditions: traditional antibiotic treatment including a single high-dosage administration event (Figure 5.A) and an innovative treatment

protocol, where preventive action occurs in parallel to the single high-dosage antibiotic administration (Figure 5.B).

We performed simulations using 50 instances of the microbiota net. As a metric to analyze the evolution of the population, we consider the simulation time[1] in which the number of human hosts in a state of severe resistance exceeds the number of human hosts in a state of mild resistance. This is a landmark of the process degeneration towards extensive resistance spread and diffusion.

In the case no treatment is administered, microbiotas do not get to a state of severe resistance ever. Therefore, a significative comparison can be only done between the traditional treatment and the innovative protocol administration conditions. In both cases, the antibiotic dosage used is equivalent to that used in the second high-dosage administration event from the previous set of experiments.

No delays are imposed over the process evolution, since in this case there is no necessity for temporal segregation of different phenomena. In fact, there is a single administration phase in the employed protocols.

When the treatment protocol provides high-dosage administration of antibiotic alone, on average the onset of a state of prevalent severe resistance within the human hosts population takes place at simulation time 162.87 ± 7.78. When antibiotic administration is provided in parallel with microbiota reintegration preventive action, on average there is a delay of 9.68%. Severe resistance onset occurs on average at simulation time 178.64 ± 7.4.

In the sample tracks of Figure 5.C and Figure 5.D, an example of the two correspondent temporal dynamics is provided. In the first example, once all the hosts population passed from an healthy state (blue) to a state of mild resistance (red), the higher probability of passing the threshold of resistance severity speeds up the population of the corresponding state (yellow). On the other hand, a much slower conversion from mild to severe resistance can be observed when the preventive action is undertaken.

IV. Conclusions

In this work, we suggested some of the potential applications of a NWN model for investigating the relations between antibiotic treatment and the spread of antibiotic resistance within the microbiota, and across human populations. A functional description of the phenomena at different levels is employed to set up a model structure, which in simulation recapitulates the system behavior under different conditions. The usage of a multi-level and hybrid formalism allowed us to integrate different kinds of data, as well as to describe multiple levels from the system under study. This is a key feature to account for the complexity of the system. In fact, we tried to encompass the problem from the level of the bacterial cells and the molecules providing them with resistance capabilities, to the single microbiota, up to the population of human hosts undergoing different treatments and interacting between each other. Simulations enabled us to take into account timing and stochastic behaviors required to represent timed administration protocols and biological processes, which are inherently stochastic processes. In this work we want to underline how models of this kind not only provide valuable tools for investigating causal relations between different events and mechanisms, but can be used as supports for decision making processes and protocol development. A complex model like ours as the potential to provide a systemic and multi-faceted view on the problem, being a valuable tool able to enrich the consideration of the problem and the testing of hypotheses or possible solutions. This model in is current form is based on strong simplifications. Nevertheless, its flexibility make it easy to adapt it to specific and more realistic use cases obtained gathering realistic data from the field.

[1] expressed as in terms of simulation cycles

V. Model availability

The full model is available at: https://github.com/sysbio-polito/nwn-microbiota-antibiotic-resistence.

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