Colonization to Infection: An Agent-Based Model of Host Response to *Mycobacterium avium* in the Lungs

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A variety of patient factors affect NTM lung infections.

Nontuberculous mycobacteria (NTM) are environmental microbes, capable of colonizing and infecting humans following inhalation of the bacteria. Estimated incidence of pulmonary disease due to NTM ranges from 4.1-14.1 per 100,000, and both incidence and prevalence of this disease have been steadily rising (1). Though exposure to mycobacteria in the environment is common, most healthy patients do not develop infections. Instead, these infections develop in more vulnerable populations with pre-existing conditions such as Cystic Fibrosis or Chronic Obstructive Pulmonary Disease.

Bacterial biofilms and phenotypes are complex and affect invasion and treatment.

These bacteria are known to form biofilms in the environment and studies have shown these biofilms to have apoptotic effects on immune cells *in vitro*. NTM biofilms have been observed in lungs of CF and COPD patients. **However, the role and dynamics of biofilms *in vivo* remains unclear**.

We integrate diverse experimental data into a simulation of infection.

We take a computational approach to explore the initial colonization of biofilm-forming mycobacteria and the associated innate immune response. Our spatio-temporal agent-based computational model includes:

- host innate immune mechanisms
  - Macrophage phagocytosis
  - Macrophage recruitment
  - Macrophage apoptosis
  - Antibacterial properties
- bacterial mechanisms
  - Growth
  - Division
  - Biofilm formation
  - Killing of host macrophages.

Model outputs include cellular and tissue scale dynamics:

- Growth and division of bacteria
- Total amount of biofilm in the site of infection
- % Macrophage death
- Spatial distribution of biofilm and bacteria

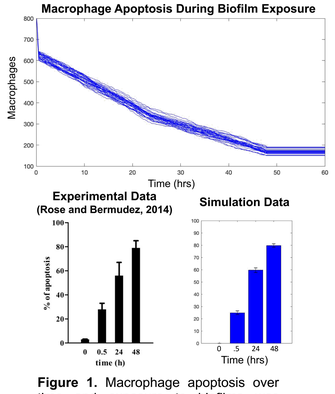
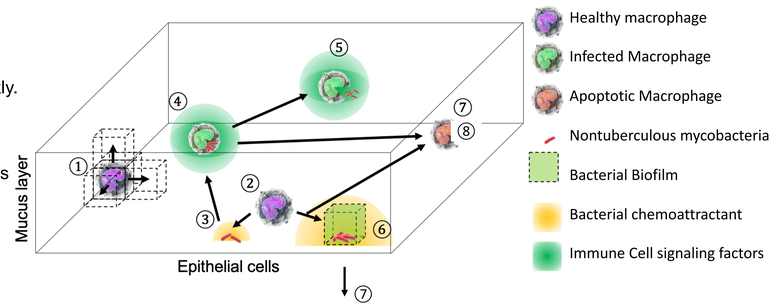


Figure 1. Macrophage apoptosis over time and exposure to biofilms was based on literature values.

Model parameters are estimated based on host-pathogen interactions and environmental measurements obtained from *in vitro* studies as well as *in vivo* from healthy and diseased patients. Initial sensitivity analysis was performed using Latin Hypercube Sampling, using narrow ranges of known parameters and broad range of unknowns parameters for 300 experiments, with 3 replicates each, for a total of 900 runs.

Simple cellular rules help us study complex tissue outcomes.

Figure 2. Model mechanisms.
(1) Healthy macrophages probabilistically follow chemoattractant gradients produced by bacteria or infected macrophages.
(2) Macrophages can phagocytose and kill bacteria near them.
(3) Planktonic bacteria do not form biofilm, but replicate more quickly.
(4) Macrophages that fail to kill bacteria become infected.
(5) Bacteria continue to replicate inside the macrophages. Once a macrophage contains too many bacteria, it bursts.
(6) Some bacteria form biofilm that protects them from phagocytosis and have apoptotic effects on macrophages.
(7) Sessile bacteria in biofilm or microaggregates may invade epithelial cells.
(8) Macrophages exposed to apoptotic signals from biofilm may apoptose.



Simulations predict cell dynamics and diverse bacterial phenotype distributions from colonization to infection.

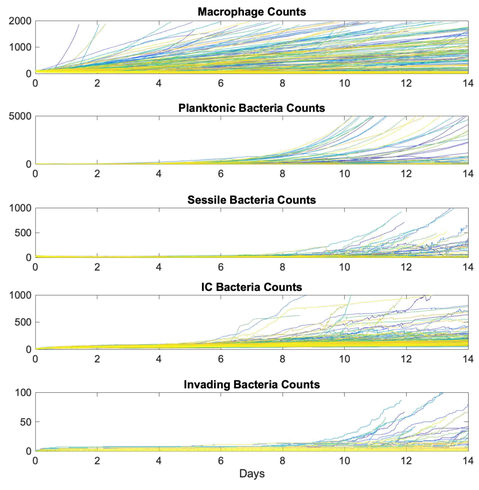


Figure 3. Representative time series of macrophage and bacterial phenotype counts (225 of 900 runs) reveal:
(a). Macrophages are consistently recruited in a linear manner, but at different rates.
(b). Planktonic bacteria grow exponentially when population is large enough, often after a delay.
(c). Sessile bacteria grow more slowly than planktonic, but can help sustain the population of planktonic bacteria.
(d). Intracellular (IC) bacteria grow and the population increases are closely tied to increases in macrophages
(e). Invading bacteria numbers are closely tied to the Sessile count.

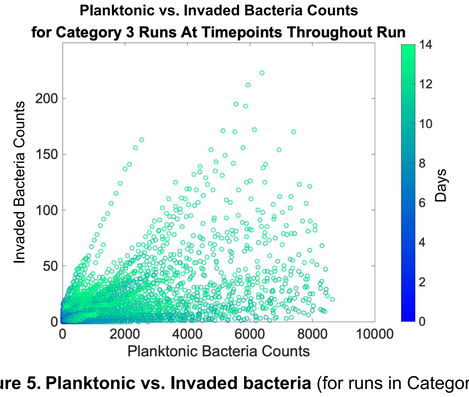


Figure 5. Planktonic vs. Invaded bacteria (for runs in Category 3 from Figure 4). Despite a dominant planktonic population, we see that invaded bacteria continue to increase throughout the run. This suggests that a sub-population of sessile bacteria remain and act as those in category 2 from Figure 4.

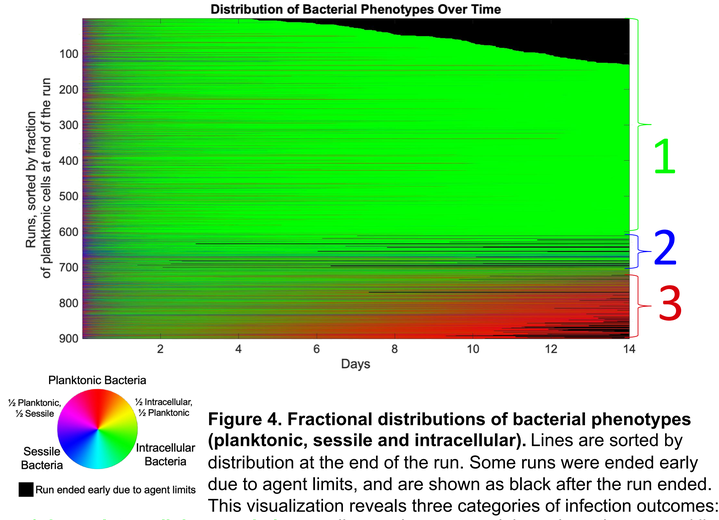


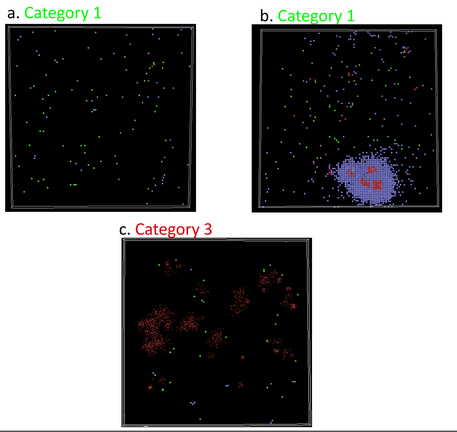
Figure 4. Fractional distributions of bacterial phenotypes (planktonic, sessile and intracellular). Lines are sorted by distribution at the end of the run. Some runs were ended early due to agent limits, and are shown as black after the run ended. This visualization reveals three categories of infection outcomes:

1. Large Intracellular population – cells are phagocytosed throughout the run, and live largely within macrophages, often leaving no extracellular bacteria. *In vivo*, these bacteria may spread to other areas of the lung through macrophages' continued movement.

2. Persistent sessile population – bacteria may be phagocytosed, but a portion remain within biofilm, where they persist due to biofilm protection from macrophages, and continue to invade epithelial cells.

3. Intracellular to planktonic – despite a large portion of cells being phagocytosed initially, the growth rate of the planktonic cells surpasses the rate of phagocytosis and the planktonic population dominates.

Figure 6. Representative images from simulation environment. (a-b) Both images show a situation with a majority of intracellular bacteria, with bacteria in (a) being distributed between a small number of macrophages, with minimal recruitment. Those in (b) have extreme recruitment in a small area, forming a granuloma-like structure. (c) Exponential growth of planktonic bacteria (red), overwhelming surrounding immune cells. Infected macrophages (green) have failed to recruit sufficient healthy macrophages.



ABMs examine host-MAC interactions, and captures a wide variety of behaviors.

- Our Agent-based model successfully replicates *in vitro*-observed interactions between *Mycobacterium avium* and macrophages.
- Our model produces a wide range of infection outcomes in mucus and epithelial invasion.
- Preliminary sensitivity analysis indicates that the role of biofilm depends on its introduction (with initial inoculum vs. produced *in vivo*) and that its influence on bacterial persistence changes over time.

Further calibration and antibiotic dynamics are in progress.

Future directions include:

- Calibrate model to replicate *in vivo* bacterial loads.
- Conduct focused sensitivity analyses with subsets of data based on different qualitative behaviors
- Add additional information regarding bacteria communication and phenotyping to explore model the sensitivity to bacterial growth rates.
- Add antibiotic dynamics to examine optimal treatment regimens

References

1. Carter, G., *et al.* J Med Microbiol (2003).

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