Abstract

Gut microbial composition has been linked to multiple health outcomes. Yet, temporal analysis of this composition had been limited to deterministic models. In this paper, we introduce a probabilistic model for the dynamics of intestinal microbiomes that takes into account interaction among bacteria as well as external effects such as antibiotics. The model successfully deals with pragmatic issues such as random measurement error and varying time intervals between measurements through latent space modeling. We demonstrate utility of the model by using latent state features to predict the clinical events of intestinal domination and bacteremia, improving accuracy over existing methods. We further leverage this framework to validate known links between antibiotics and clinical outcomes, while discovering new ones.

1 Introduction

The human intestinal environment is host to a variety of microbial organisms. This community is an essential component of human health [3]. Microbiome composition has been associated with increased risk for multiple common diseases, among them type 2 diabetes [9], obesity [6], and Crohn’s disease [10]. Nonetheless, the complex interactions between host, environment, and external perturbations are just beginning to be understood [2, 4].

Recent advances in metagenomic and targeted DNA sequencing, as well as reduced cost, have allowed researchers to investigate the microbiome in unprecedented detail [7]. However, the utility of this data in a clinical setting remains under-explored [12]. Previous work has focused on developing deterministic or descriptive approaches to establish the role of the microbiome in providing resistance to infection, e.g. [13]. Intestinal domination (an imbalance in community composition favoring one taxon) of Enterococcus, Streptococcus, or Proteobacteria were common complications, which further increased the risk of bacteremia in each patient. Yet, these approaches ignore randomness in the underlying system as well as measurement noise, and therefore provide limited capacity to predict patient outcomes. In contrast, probabilistic approaches provide a natural way to model uncertainty, and provide a systematic way to make predictions.

Here we develop a probabilistic time-series model of microbiome community dynamics. Our goals are three-fold: i) incorporate uncertainty in the ascertainment process, ii) develop a model capable
of capturing interactions within the community over time, and; iii) explicitly model external effects such as antibiotics. We test our model on a data set of 94 patients of various cancers (majority Leukemia and Lymphoma) undergoing allogenic hematopoetic stem cell transplant (allo-HSCT). Two common complications of this procedure are bacteremia, a bacterial infection of the blood, and intestinal domination, defined to be the event that relative abundance of one bacteria was greater than 30%. We demonstrate that by incorporating randomness into the time-series analysis of microbial community dynamics, we can more accurately predict patient outcomes such as bacteremia and intestinal domination, than using community composition alone.

2 Methods

2.1 A Latent Probabilistic Model Based on Kalman Filters

We observe DNA read counts from each bacterial taxon, while the true community composition — the relative abundance of each taxon in a patient sample — is latent. We model temporal dynamics of the community as latent factors of a linear dynamical system \( \mathcal{M}(A, B, Q, Q_0, R, \mu_0) \) is

\[
\begin{align*}
    z_0 & \sim N(\mu_0, Q_0) \\
    z_{t+1} | z_t & \sim N(Az_t + Bu_t, Q) \\
    x_t | z_t & \sim N(z_t, R)
\end{align*}
\]

(1) (2) (3)

Here, time \( t \) is measured in days. Tomorrow’s vector representing each species’ abundance, \( z_{t+1} \), depends on today’s composition \( z_t \), interactions between pairs of taxa (the matrix \( A \)), the antibiotic dosage \( (u_t) \), and the effect on antibiotics on each taxon \( (B) \).

Observations \( x_t \) pose several challenges. First, they are not daily, nor evenly spaced, but we still model days as latent \( z_t \), even if observations are available only for a subset \( S \) of days. Further challenging is data only available in relative abundances, listing the frequency of each taxon at a time step, not the absolute count of cells. Thus, observation vectors are constrained to the positive \( L^1 \) unit sphere, making time series modeling difficult. We investigated several data transformations to infer through this constraint, and decided to use the centered log-ratio transform (clr) \( ^8 \): the vector \( y = (y[1], \ldots, y[D]) \) of relative abundances of \( D \) taxa, is log-transformed and mean-shifted:

\[
    \text{clr}(y) = \left( \log \frac{y[1]}{\prod_{i=1}^D y[i]^{1/D}}, \ldots, \log \frac{y[D]}{\prod_{i=1}^D y[i]^{1/D}} \right)
\]

(4)

We map \( y \) to a modeled observation \( x \in \mathbb{R}^{D-1} \) as \( x = (\text{clr}(y)[1], \ldots, \text{clr}(y)[D-1]) \). This mapping is invertible as

\[
    y = \text{normalize}\left( (e^{x[1]}, \ldots, e^{x[D-1]}, e^{-\sum_d x[d]}) \right)
\]

(5)

We learn parameters \( A, B, Q, Q_0, R, \mu_0 \) using an EM algorithm \( ^{5} \). Given this formulation, we can still compute both marginal conditional expectations of \( z_t \) in the E step and closed form expressions for the optimal model parameters in the M step regardless of missing daily observations, in the transformed, unconstrained space.

2.2 Prediction of Clinical Events

To predict the clinical events of bacterial domination and bacteremia, we trained our Kalman Filter model on other samples (50X cross-validation), and fit a linear logistic classifier \( ^{11} \). The classifier’s input was latent composition vectors predicted by the model for the time of the event. This was compared against a baseline of predictions based on the most recent observation of a composition vector. We repeated these predictions using composition vectors either in original frequency space, or as clr-transformed vectors. For domination of a particular taxon, we further explored regressing only on the abundance of that taxon.

We considered several predictions of clinical events of bacterial domination and bacteremia. Predictions used logistic regression. We repeated these predictions using either the clr-transformed vectors or the abundances.
Figure 1: Receiver-Operator Curves (ROC) demonstrating performance of our classifier on predicting intestinal domination by Enterococcus (left) Streptococcus (middle) and Proteobacteria (right). We compare the classifiers built by: (1) clf transformation (solid) vs. original frequency (dashed) vs. frequency of that bacteria alone (dotted) ; and (2) Kalman Filter prediction (green) vs. Most recent observation (red)

Figure 2: ROCs demonstrating performance of our classifier on predicting bacteremia due to VRE (left), gram-negative bacteria (middle), or all (right). Dashed/solid and color conventions follow Figure 1.

For evaluating the contribution of antibiotics to clinical events, we forward simulated the model starting from each observation time point for 10 days. We reported the simulation with or without the drug under consideration and registered the reported probabilities for the clinical event.

2.3 Data and availability

439 measurements of gut microbiome composition were collected across 94 subjects undergoing allo-HSCT [14] followed by a regimen of antibiotics. Measurements were spaced 1-21 days (median: 7), and spanned a period of 0-13 days (median: 6) before and 7-35 days (median: 18) after the transplantation. Measurements were taken by extracting DNA from fecal specimens amplifying the V1-V3 region of the 16S rRNA genes, and phylogenetic classification of each sequence performed at the genus level (see [14] for more details).

3 Results

We first sought to predict which patients will develop intestinal domination of one of the above bacteria. Our Kalman Filter predictions of bacterial domination (Figure 1) consistently exceed baseline performance. Specifically, Enterococcus domination is predicted by the Kalman Filter with Area Under Curve (AUC) of 0.78, while that of most recent observation was 0.77. Similar improvements were demonstrated for Streptococcus (0.66 vs. 0.59) and Proteobacteria domination (0.72 vs. 0.63). Reassuringly, the best Kalman Filter prediction prediction was always the one based on the dominant taxon only.

We separately fit classifiers for the outcome variable of bacteremia, either due to Vancomycin-resistant Enterococcus (VRE), or by gram-negative bacteria. Again, our model outperformed our baseline
Figure 3: Effect of antibiotics on intestinal domination and bacteremia. a) Metronidazole increases the risk of Enterococcus domination. Fluoroquinolone reduces the risk of Proteobacteria domination, and Beta-lactum reduces the risk of Streptococcus domination. b) Metronidazole increases the risk of VRE bacteremia and gram-negative bacteremia.

measure (Figure 2). AUCs for Kalman Filter predictions were 0.83 (VRE bacteremia), 0.70 (gram-negative bacteremia) and 0.68 (any bacteremia), compared to the AUCs for best MRO classifiers (0.76, 0.69 and 0.58, respectively).

We next turned evaluating the contribution of antibiotics to bacterial domination and bacteremia (see Section 2). For each observation timepoint, we plot the evaluated probability of the clinical event occurrence with vs. without a particular antibiotic drug (Figure 3). Our analysis of domination is qualitatively consistent with previous findings [14]: Metronidazole is significantly positively associated with Enterococcus domination, increasing the probability of domination by 25.7 percentage points (pp) on average and (1.6-fold); Fluoroquinolone is negatively associated with Proteobacteria domination, decreasing the probability by 19.7 pp and 1.46-fold. We observe that these fold change evaluations are lower than those of [14], whose respective point-estimates are 3-fold and 10-fold. We further discover a strong association between Beta-lactam and Streptococcus domination, whose domination probability it reduces by 23.7 pp (1.41-fold).

An analogous analysis of bacteremia highlights Metronidazole. As expected from its association with Enterococcus domination, it increases the risk of VRE bacteremia 1.9-fold. Our model further discovers Metronidazole to be positively associated with gram-negative bacteremia, increasing its probability 1.4 fold. This is an improvement over the previous study [14] which used survival analysis only and did not detect the effect of the beta-lactam antibiotics on the decreasing risk of Streptococcus domination.

4 Discussion

In this paper, we have developed a probabilistic model for microbiome community dynamics that explicitly incorporates measurement error and external effects such as antibiotics. We demonstrated the utility of this approach by applying our model to data from real patients, and showed that incorporating time-series information leads to better predictions for patient outcomes. Finally, we used this framework to discover links between antibiotics and clinical outcomes, validating discoveries against published results.

Kalman Filter essentially attempts to optimize estimation of the latent composition vectors, minimizing RMSE under the appropriate transformation [8]. It can therefore predict composition well under this transformation, but not without it (data not shown). Instead, we focused on predicting outcomes. These seem more robust to transformations, and preserve the utility of the prediction.

Nonetheless, there is still room for improvement to our model. The predicted microbiome composition, though informative, is a noisy estimate at best (data not shown). This is likely due to either data sparsity in time — our observations are several days apart, or data size — we only have 94 patients.
Either of these could be remedied using denser data with more patients, which are likely to be available soon.

References

[1] Christopher M Bishop. Pattern recognition and machine learning. Springer, 2006.

[2] J Gregory Caporaso, Christian L Lauber, Elizabeth K Costello, Donna Berg-Lyons, Antonio Gonzalez, Jesse Stombaugh, Dan Knights, Pawel Gajer, Jacques Ravel, Noah Fierer, et al. Moving pictures of the human microbiome. Genome biology, 12(5):R50, 2011.

[3] Jose C Clemente, Luke K Ursell, Laura Wegener Parfrey, and Rob Knight. The impact of the gut microbiota on human health: an integrative view. Cell, 148(6):1258–1270, 2012.

[4] Lawrence A David, Arne C Materna, Jonathan Friedman, Maria I Campos-Baptista, Matthew C Blackburn, Allison Perrotta, Susan E Erdman, and Eric J Alm. Host lifestyle affects human microbiota on daily timescales. Genome Biology, 15(7):R89, 2014.

[5] Arthur P Dempster, Nan M Laird, and Donald B Rubin. Maximum likelihood from incomplete data via the em algorithm. Journal of the Royal Statistical Society. Series B (methodological), pages 1–38, 1977.

[6] Harry J. Flint. Obesity and the Gut Microbiota. Journal of Clinical Gastroenterology, 45(December):S128–S132, 2011.

[7] Justin Kuczynski, Christian L Lauber, William A Walters, Laura Wegener Parfrey, José C Clemente, Dirk Gevers, and Rob Knight. Experimental and analytical tools for studying the human microbiome. Nature Reviews Genetics, 13(1):47–58, 2012.

[8] Zachary D. Kurtz, Christian L. Müller, Emily R. Miraldi, Dan R. Littman, Martin J. Blaser, and Richard A. Bonneau. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. PLoS Computational Biology, 11(5), 2015.

[9] Nadja Larsen, Finn K. Vogensen, Frans W J Van Den Berg, Dennis Sandri Nielsen, Anne Sofie Andreassen, Bente K. Pedersen, Waleed Abu Al-Soud, Søren J. Sørensen, Lars H. Hansen, and Mogens Jakobsen. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS ONE, 5(2), 2010.

[10] Xochitl C Morgan, Timothy L Tickle, Harry Sokol, Dirk Gevers, Kathryn L Devaney, Doyle V Ward, Joshua A Reyes, Samir A Shah, Neal LeLeiko, Scott B Snapper, Athos Bousvaros, Joshua Korzenik, Bruce E Sands, Rami J Xavier, and Curtis Huttenhower. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biology, 13(9):R79, 2012.

[11] Fabian Pedregosa, Gaël Varoquaux, Alexandre Gramfort, Vincent Michel, Bertrand Thirion, Olivier Grisel, Mathieu Blondel, Peter Prettenhofer, Ron Weiss, Vincent Dubourg, et al. Scikit-learn: Machine learning in python. Journal of Machine Learning Research, 12(Oct):2825–2830, 2011.

[12] Eamonn M. M. Quigley. Gut microbiota as a clinical tool in gastrointestinal disease management: are we there yet? Nature Reviews Gastroenterology & Hepatology, 14(5):315–320, mar 2017.

[13] Richard R. Stein, Vanni Bucci, Nora C. Toussaint, Charlie G. Buffie, Gunnar Rütsch, Eric G. Pamer, Chris Sander, and João B. Xavier. Ecological Modeling from Time-Series Inference: Insight into Dynamics and Stability of Intestinal Microbiota. PLoS Computational Biology, 9(12), 2013.

[14] Ying Taur, Joao B Xavier, Lauren Lipuma, Carles Ubeda, Jenna Goldberg, Asia Gobourne, Yeon Joo Lee, Krista A Dubin, Nicholas D Socci, Agnes Viale, Miguel-Angel Perales, Robert R Jenq, Marcel R M van den Brink, and Eric G Pamer. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 55(7):905–14, oct 2012.