

# Global Interactions & Disease Drivers of the Human Virome

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# Abstract

Here we present a global view of phage-bacteria interactions across the human virome. We present our model for phage-bacteria interactions with validation for accuracy and sampling coverage. These networks are valuable because they do not rely on the sub-optimal reference genome datasets, and provide a more accurate view of the relationships within the community. We find that interactive dynamics are associated with disease states and anatomical body sites, using a global virome meta-analysis dataset. Our comprehensive approach to understanding the virome provide new insights not only into composition and diversity, but their context in the greater community. We find that disease states and anatomical sites are not only linked to altered community composition and diversity, but also represent significant shifts in interactive dynamics.

## Introduction

The human virome is a crucial component to the human microbiome, and consists largely of bacteriophages (bacterial viruses). Because phages lack metabolic capabilities, they rely on hijacking their bacterial hosts to perform their metabolic tasks. In this sense, the impact of phages is dependent on their infectious interactions with their bacterial hosts, which can include community composition modulation through predation and lysis, or impact on bacterial functionality through altered gene expression or horizontal gene transfer (transduction). To date, analysis of the human virome has focused annotation techniques largely adopted from bacterial metagenomic methods, but the virome system is different due to the inability of phages to act on their own. By incorporating system-wide infectious interactions into our analyses, we can begin to gain a more complete understanding of the human virome.

One such barrier is the dismal state of contemporary virus reference databases. The majority of phage genomes are predicted to be unannotated, and the existing phage reference genomes have been isolated on bacteria from only 3 phyla (cite sullivan maybe).

The microbiome is a primary driver in global chemical processes, including environments as diverse as the ocean and the human body. Organisms such as bacteria perform metabolic processes but are controlled in large part by the associated virus community (the virome). Viruses control microbiome metabolism through predation, gene expression control, and horizontal gene transfer. In humans, this translates into virus control of disease and metabolism. Despite being crucial members of the human microbiome, we understand remarkably little about the human virome.

In addition to studying specific virome cohorts, it will be important to investigate the global trends associated with these communities. Such large scale virus studies have proven valuable for understanding the global marine virome, and we are leveraging a similar approach here for the human virome (cite sullivan science and hurwitz pnas). This view from ten thousand feet provides us with a new tier of understanding the ecology of the human virome, and how it relates to human metabolism and disease.

## Results

### The Global Human Virome Dataset

We leveraged the extensive public sequence archives to assemble a global human virome dataset; a robust human virome dataset that spans diverse body site environments. Our sampling includes the gut, oral cavity, skin, and urinary tract systems, all of which are associated with healthy and disease states, and were all collected by multiple, independent groups. We only worked with virome datasets that were purified for virus like particles (VLPs) before sequencing. This provides us with greater analytical strength and more power conclusions about the observed virus biology. The resulting dataset contains data from ten virus metagenomic studies<sup>1-9</sup>.

### Sampling Depth and Dataset Validity

Because the virome sequence space is dominated by unknown and poorly annotated genomes, we focused on the Operational Protein Families (OPFs) within the assembled contigs of the dataset. Operational protein families are functionally similar to operational taxonomic units, in that they are groups of open reading frames with similar nucleotide sequences. Here I also need to make sure I outline why core OPFs are important.

We began by assessing the current progress in sampling the human virome OPF space. A rarefaction analysis revealed that virome OPFs can be sufficiently covered in a single study, however the average sequencing depth to achieve such coverage is

relatively high at approximately 10 million sequences (**Figure 1 A**). We quantified the rarity of the OPFs by showing that the majority of OPFs only had sequence counts between 10-1000 (**Figure 1 B**). These results provide important information that inform both the technical and biological aspect of the human virome. Technically, the general sequencing depth for a human virome study should be approximately 10 million sequences. At this point the majority of OPFs have been sampled. Biologically, this lends confidence to our current understanding of the virome because we are able to sample the majority of virome operational protein families.

We investigated the core and pan viromes by calculating the shared distribution of phage OPFs across the samples. As expected, the core virome tapers off rapidly as the sequencing depth increases. Individual body sites harbor different core viromes. The majority of the core genes were housekeeping and structural genes, however we did identify some auxiliary metabolic genes throughout the human virome. Auxiliary metabolic genes are phage-encoded genes that convey a bacterial or eukaryotic function. These genes represent the tools that phages use to manipulate their bacterial host population functionality.

## Modeling Phage-Bacteria Interactions Across the Human Virome

We used Neo4J graph database software to construct a network of predicted interactions between bacteria and bacteriophages. Results from a variety of complementary interaction prediction approaches were layered into a single network (**Figure 1**). *In vitro*, experimentally validated interactive relationships were taken from the existing literature. Clustered Regularly Inter-spaced Short Palindromic Repeats (CRISPRs) are a sort of bacterial adaptive immune system that serves as a genomic record of phage infections by preserving genomic content from the infectious phage genome. These records were used to predict infectious relationships between bacteria and phages. Infectious relationships were also predicted by identifying expected protein-protein interactions and known interacting protein domains between phages and their bacterial hosts. We finally used nucleotide blast to identify genomic similarity between bacteriophage genomes and sections of bacterial genomes. Such a match is a good predictor of an interaction between the phage and it's bacterial host.

We validated our predictive graph model by quantifying the sensitivity and specificity using a manually curated dataset of experimentally validated positive and negative interactions. Experimental results were extracted from manuscripts published between 1992 and 2015 (**Figure 3**)<sup>10-15</sup>. This allowed us to both evaluate the utility of the model, as well as determine the optimal decision thresholds to use for predictions.

The resulting model had an AUC of 0.651, an optimal sensitivity of 0.872, and an associated optimal specificity of 0.517 (**Figure 4**). We are therefore able to effectively avoid false positive interactions, while at the same time detecting the minimum amount of interactions within the system.

## Interactive Dynamics Are Associated with Anatomical Sites

Phages are known to transfer genetic content between bacteria in the process of transduction. This has great medical importance when considering transduction of antibiotic resistance genes and other virulence factors. In a dense microbial community, transduction is likely to play an important role in bacterial fitness and virulence. To date, we have a minimal understanding of the interactions phages are facilitating between bacteria. Furthermore, the roles of broadly infecting phages have yet to be considered. Our graph approach allows us to begin predicting and understanding these interactions.

We predicted the phage-mediated relationships between bacteria by executing triadic closures as (bacteria)-[phage]->[bacteria]. Triadic closure theory states that a strong relationship of two entities to a shared intermediate suggests a relationship between the two previously unrelated entities. In our case, we are assigning relationships between bacteria based on shared strong relationships to a phage intermediate.

One of the most powerful aspects of this analysis is that it allows us to evaluate the global interactive properties of the interactive networks across the body and thus provide insight into the complex ecological dynamics. We found that the phage-bacteria interactive network follows a scale-free distribution instead of a random exponential distribution. Not only does this indicate a lack of randomness in the population, it also suggests the hub is composed of hubs that are highly interconnected to the remaining nodes.

## Disease States Drive Altered Interactive Network Dynamics

The virome has been associated with a variety of disease states across many body sites. Because many of the virome samples within our global virome dataset were associated with diseases, we were able to identify and confirm global virome trends in the human virome. We found that the diversity of disease samples was impacted by the body site. Despite the disease, the body site contributed to the virome diversity signature.

Here I want to get at the fact that at first, given a stable bacterial reference, the interactive dynamics of the networks differ between disease and healthy states.

I can use eccentricity centrality to define the most central microbial nodes of the complex graph.

The diameter of the network is short, suggesting a small-world distribution. Because it follows a scale-free distribution, it is also protected from random attack, but highly susceptible when hub nodes are impacted. I will need to expand on this later.

## Discussion

An application that we alluded to here is a graphical approach to microbiome research in general.

## Materials & Methods

## Figures

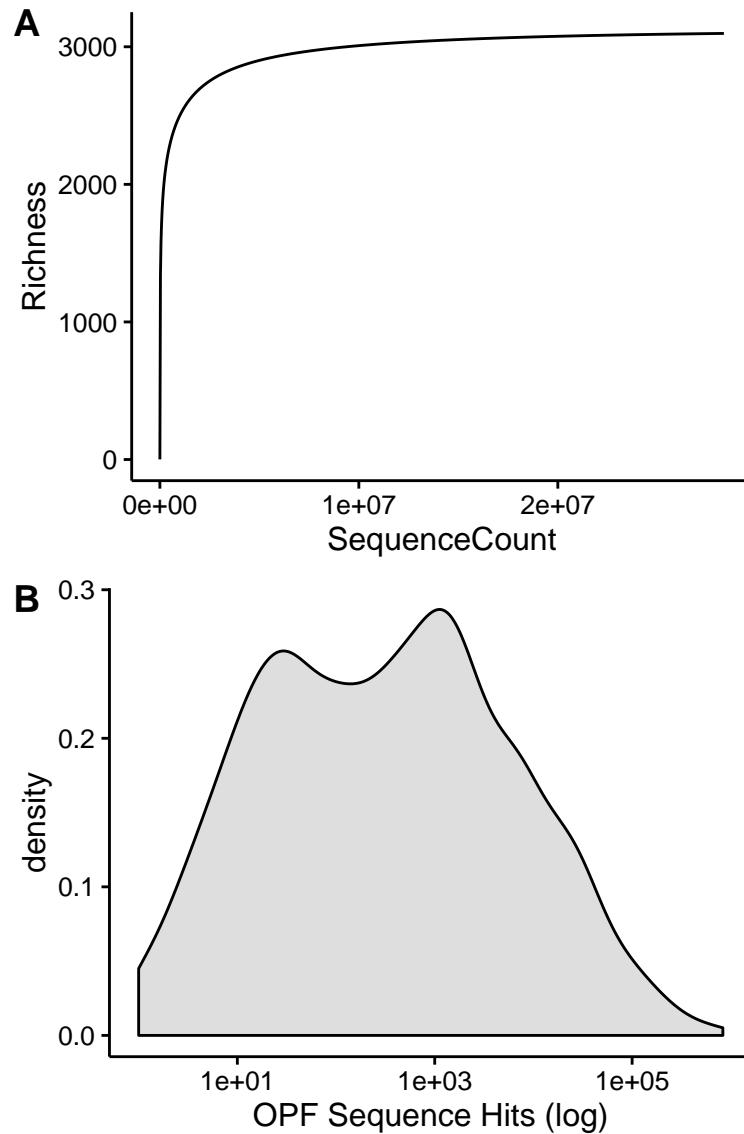


Figure 1: Analysis of sequencing coverage required to sufficiently sample the human virome. A) Rarefaction analysis of the number of OPFs detected (richness) as more sequences are used from the dataset. B) Distribution of the number of sequences that mapped to each OPF.

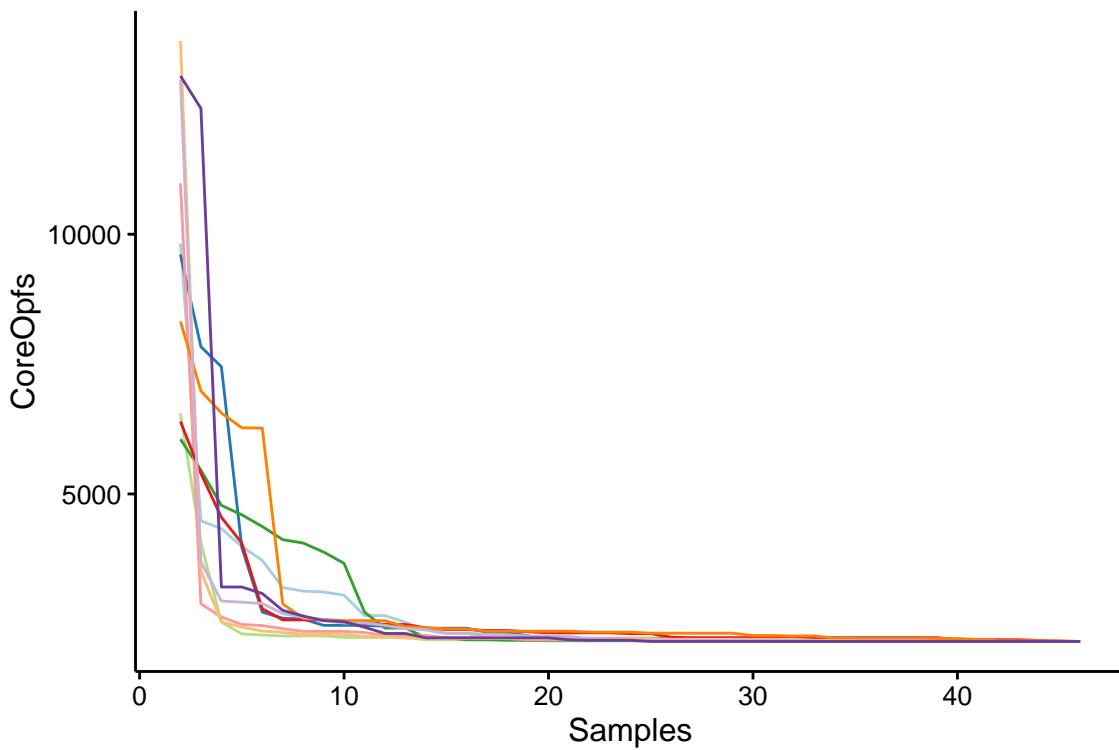


Figure 2: Rarefaction of decreasing core OPFs in the human virome with increasing samples. Colors represent iterative permutations using randomly added samples in different orders.

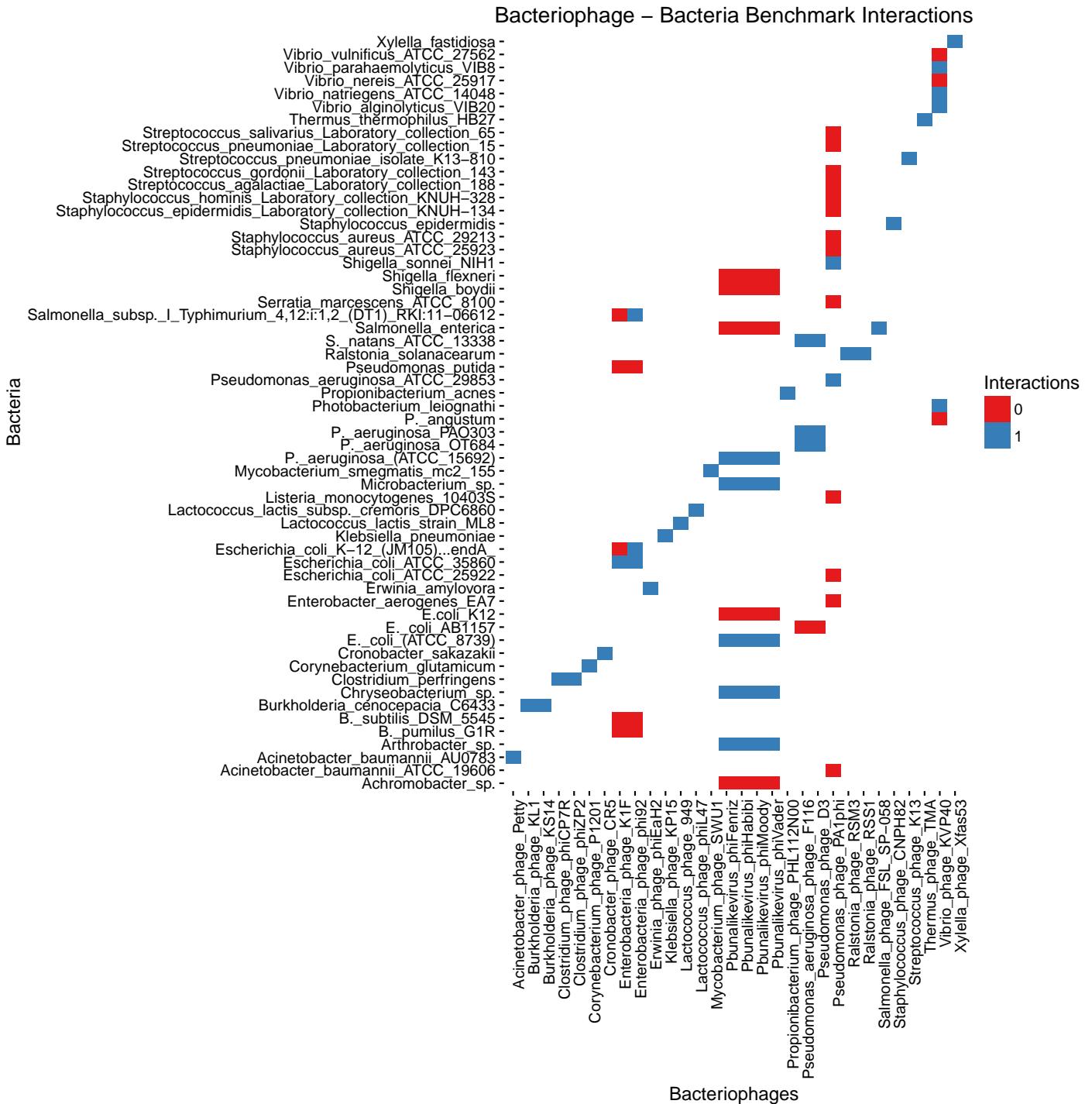


Figure 3: Positive and negative interactions of our reference dataset.

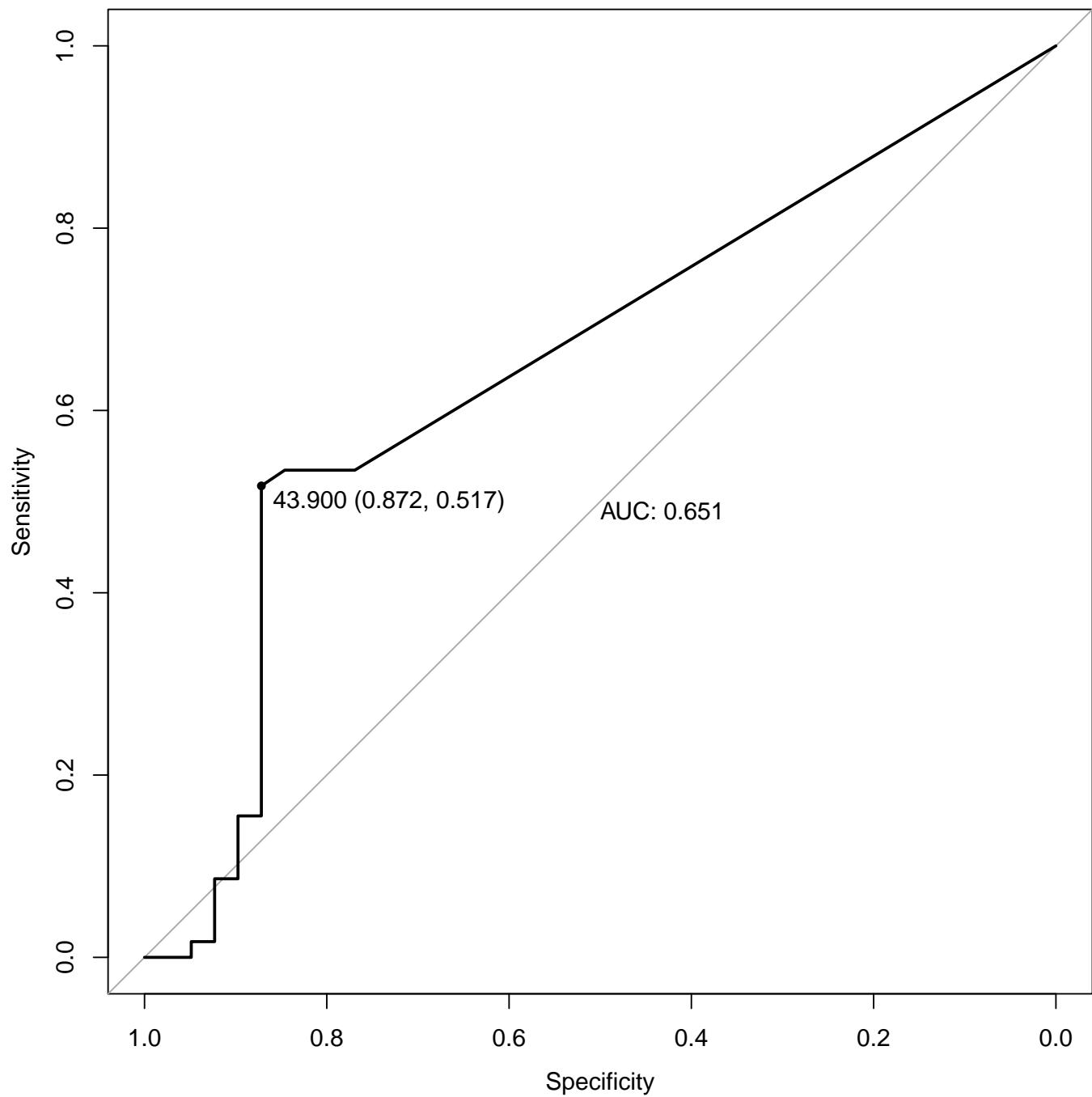


Figure 4: ROC curve used to validate the graph model of phage-bacteria interactions.

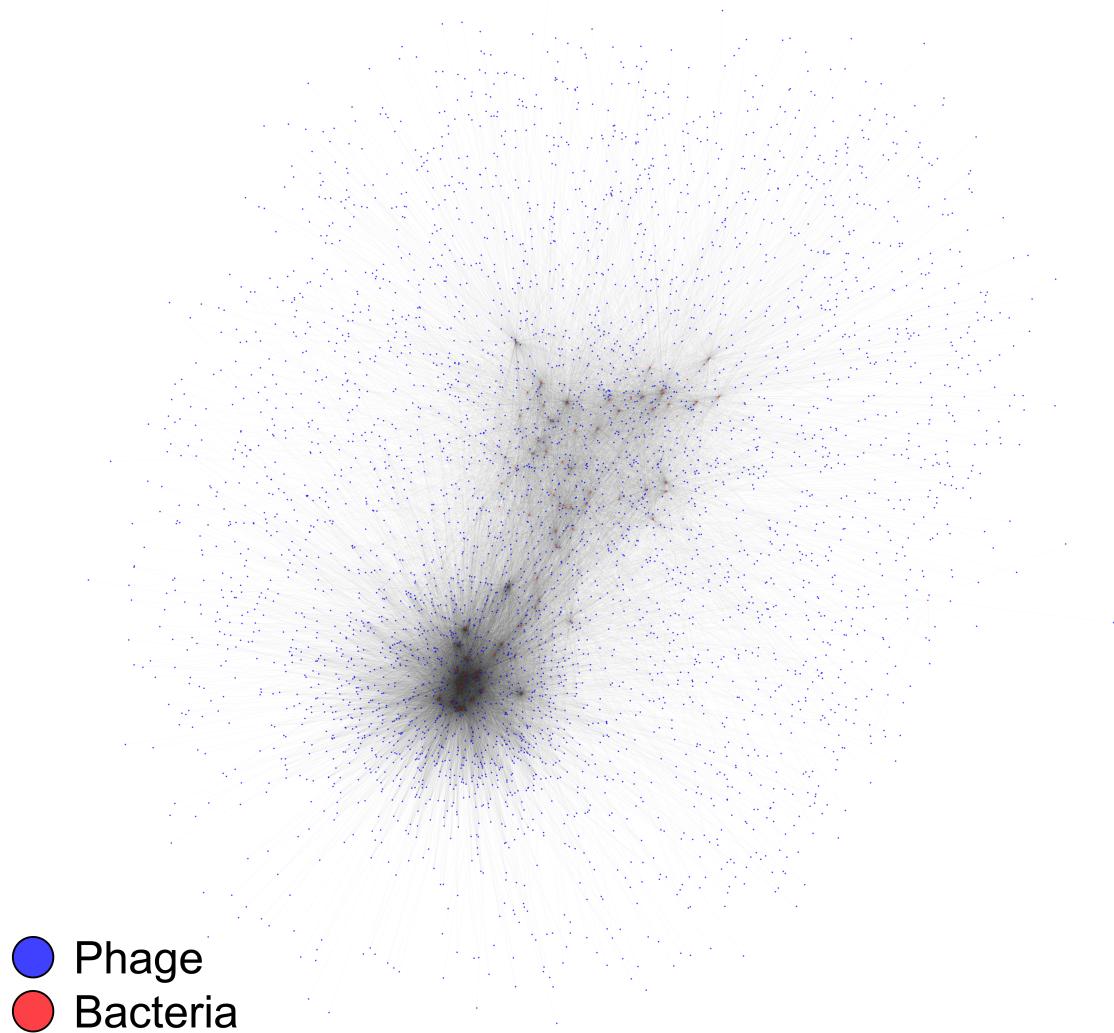


Figure 5: Network diagram of the phage - bacteria network.

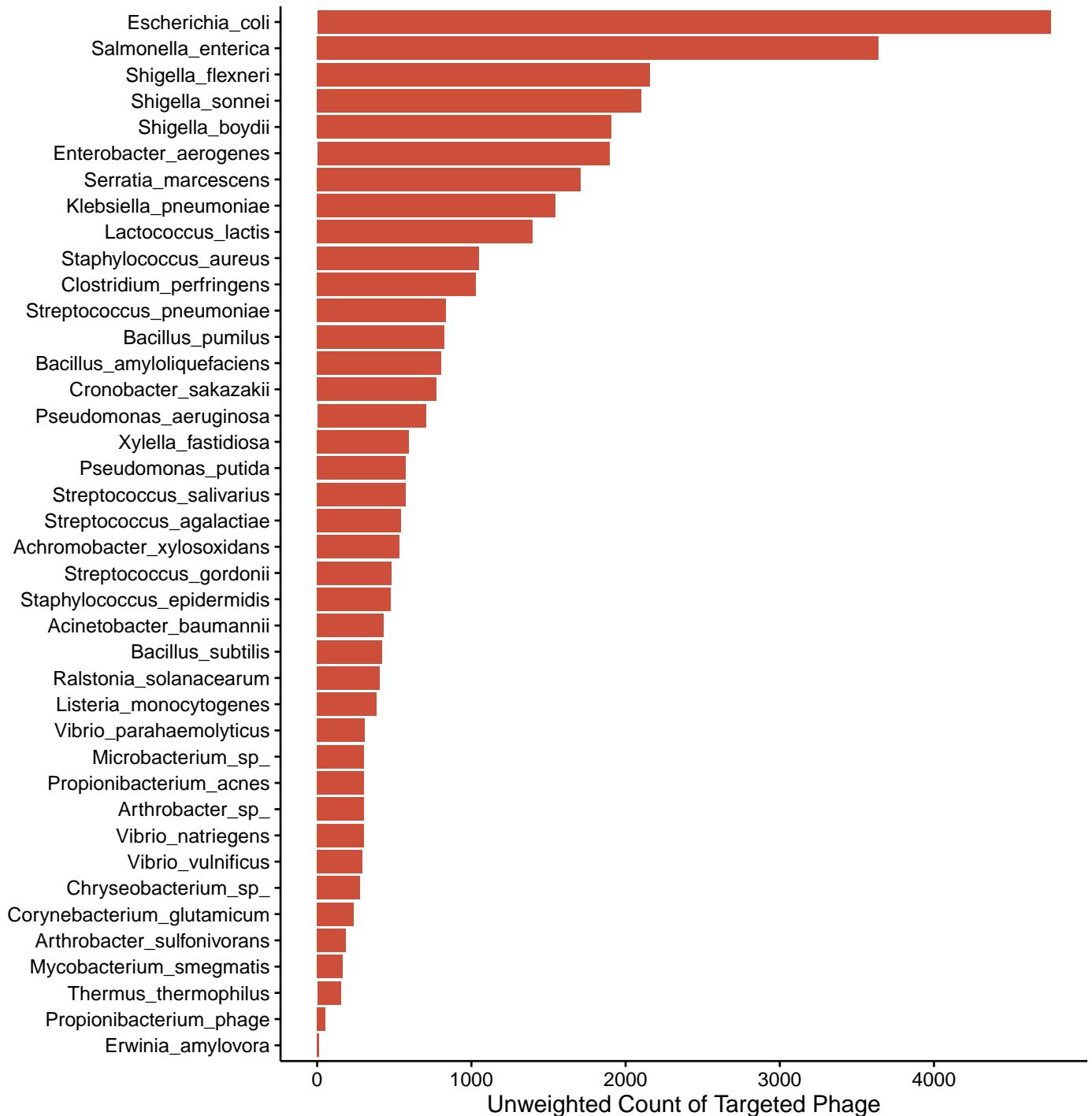


Figure 6: Relative abundance presence of phages by their predicted host target.

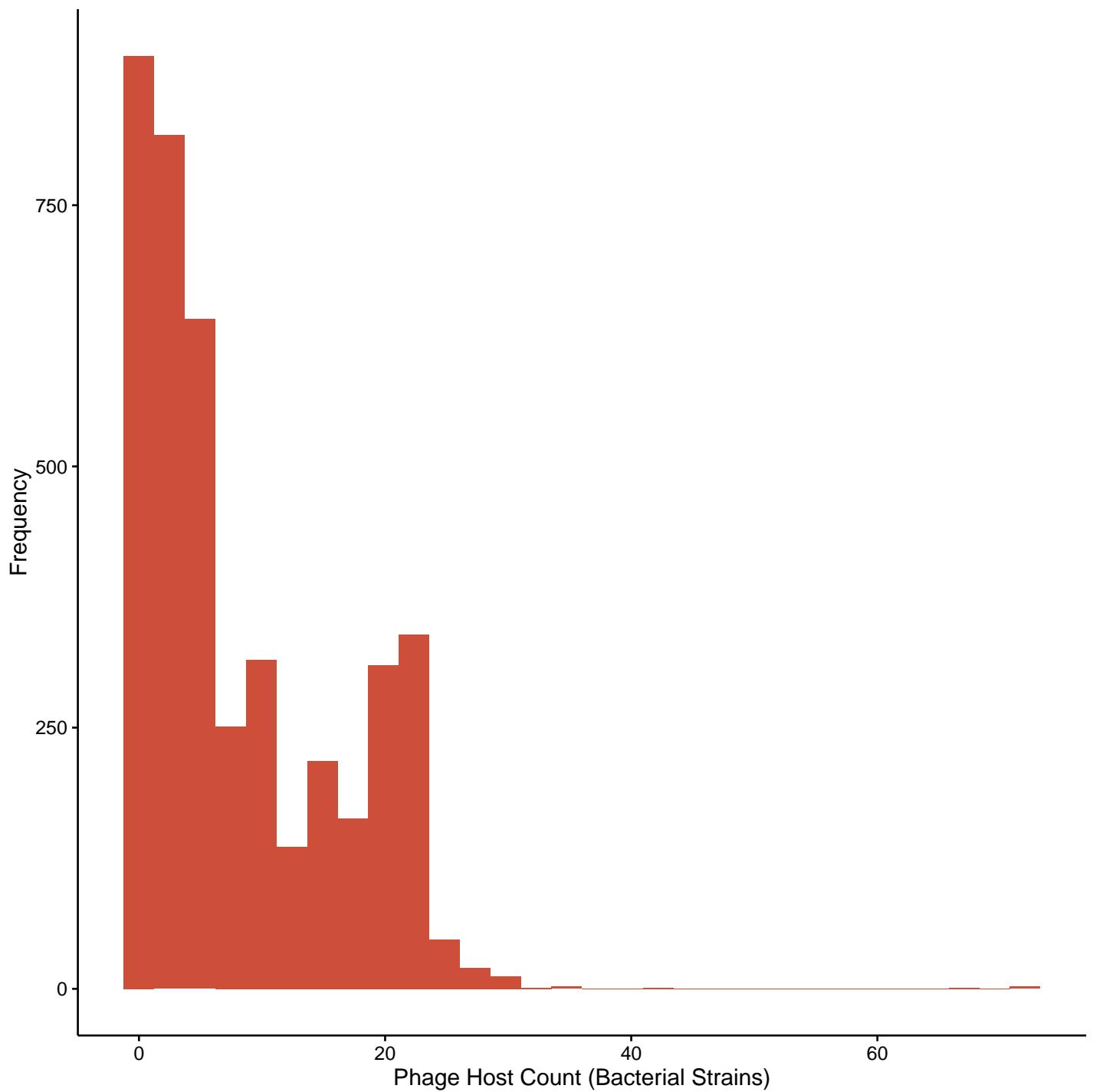
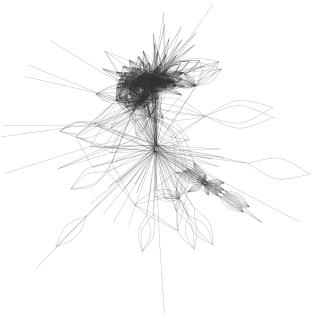


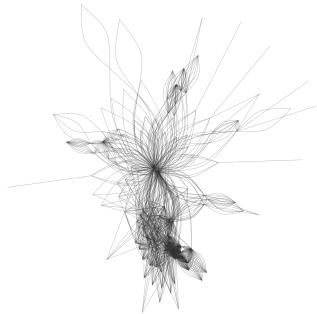
Figure 7: Histogram of the number of bacterial strain hosts identified for each phage contig.

**`Crohn's\_disease`**



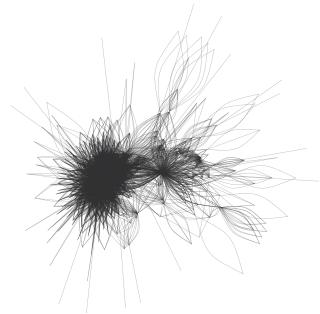
● Phage  
● Bacteria

**`Household\_control`**



● Phage  
● Bacteria

**`Ulcerative\_colitis`**



● Phage  
● Bacteria

Figure 8: Network visualization for each disease category.

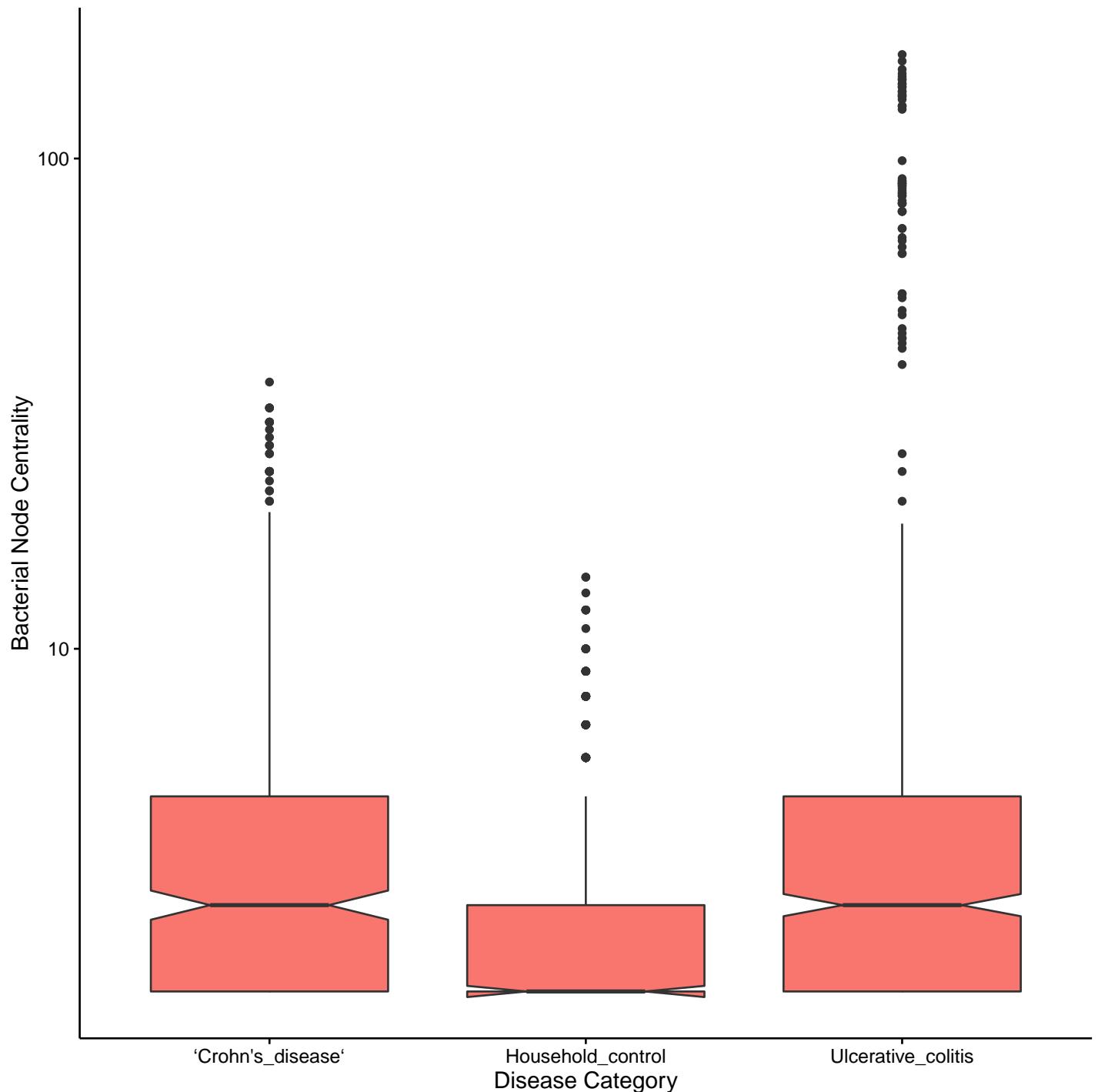


Figure 9: Bacterial centrality across disease states.

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