

**DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY**

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Dear Editor,

We submit for your review: “***Clostridium difficile alters the structure and metabolism of distinct cecal microbiomes during initial infection to promote sustained colonization***” by Matthew Jenior and our colleagues. Because this study integrates microbial ecology, classical pathogenesis, and novel computational techniques to understand the interaction of a clinically relevant pathogen with perturbed microbial communities, we feel that this work is firmly within the scope of *mSphere*.

*C. difficile* infection has grown to be one of the most prevalent causes of hospital acquired infection in the United States. Understanding the specific factors that allow for infection is a critical step in ultimately designing targeted therapeutics against *C. difficile*. Susceptibility to *C. difficile* infection is caused by recent treatment with antibiotics. Aside from their beneficial effects, these drugs are known to change the structure of the gut microbiome as well as the availability of growth nutrients in the gut. We performed 16S rRNA gene sequencing, toxin assays, metabolomics, and metatranscriptomics to study the interaction between the gut microbiota and *C. difficile* as it colonized the cecum of antibiotic-treated mice. For the first time, our manuscript reports how the available niche space is expanded following antibiotic perturbation allowing *C. difficile* to colonize. In addition, it provides a first look at how the community responds to *C. difficile* during colonization. By combining a multi-omics approach with community ecology, we were able to demonstrate that although clinidamycin changed the community structure of the gut microbiota, the effect on the metabolic activity and gene expression was minimal and accounts for the ability of the community to clear *C. difficile* after it initially colonized. Although we do not address this directly, our work indicates that devising a therapy that consists of a single strain is unlikely to work because *C. difficile* is a generalist and will be able to adapt its physiology to the available niche space. Our work emphasizes the need to focus on the overall physiology of the resident community to understand why *C. difficile* is able to colonize. We hope that you agree that this is an important contribution to the literature.

The data presented in this manuscript are original and the manuscript is not under consideration elsewhere. The manuscript was previously reviewed at *PLOS Pathogens* and rejected, after revising the manuscript and responding to the reviewer comments we submitted the manuscript to *mSphere* (mSphere00083-18) for an expedited review. Unfortunately, the editor did not feel that our responses were sufficiently thorough and the manuscript was rejected. Since receiving that decision, we have completed a thorough editing of the manuscript and are now resubmitting the manuscript. None of the manuscript contents have been previously published. The manuscript has been deposited on the bioRxiv pre-print server. All authors have read and approved the manuscript for publication. The authors declare no conflicts of interest.

Unfortunately, we must request that **Rob Knight (UCSD)** not be included as an editor or reviewer of this manuscript as previous interactions indicate that he is unable to provide an unbiased review of our work.

Thank you very much for your consideration and we look forward to your response.

Sincerely,

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