**Reviewer #1 (Comments for the Author):**

**Overview of the study:**

**The authors have described a multi-omics approach including 16S rRNA microbiome characterization and metagenome-enabled metatranscriptomics to get a broad understanding of the *Clostridium difficile* infection in relation to the indigenous tissue associated microbiome structure, changes in metabolomes and *C. difficile* clearance rate.**

**Importance:**

**This is a very comprehensive study that can give an insight into understanding response to antibiotics on microbiome structure and role of metabolomes during *C. difficile* infection. Furthermore, the study can help design better therapeutics and also help moderate the use of antibiotics. Here are some minor questions and suggestions for the authors.**

**Questions:**

**1) One of the findings was that the “composition of streptomycin-pretreated communities was more variable between cages”, are these mice related (example being from same litter) within a cage and in this case was the genetic relatedness (IBD) corrected for?**

This is an excellent point, and we apologize for the omission within the Methods section. Mice within each experimental group were primarily littermates whenever possible. Only a very small subset of animals in a given group were not from the same litter, strictly to balance numbers. This has now been expanded on in the Methods.

**2) Did strains of *C. difficile* remain same between the cages post 18 hours infection period?**

We utilized ribo-type characterization of the single spore stock used for all experiments and Sanger sequencing of the full length 16S rRNA gene from *C. difficile* grown on plates after infection to ensure we were still working with the same organism. These results all remained consistent, making us confident we were dealing with the same organism at the beginning and end of experiments. Although these specific methods do not entirely preclude the possibility of some strain variation, it is very unlikely. Additionally these mice were maintained under a strict husbandry protocol, so while not impossible, it is unlikely that an additional strain of *C. difficile* would have been introduced during the course of the experiments.

**3) Did the authors look at the presence/prevalence of any previously identified antibiotic resistant genes in the microbiome community? Did they find any of the integron-integrase gene classes?**

While this was not the focus of the current study, this is an excellent question and could provide and explanation for the underlying differences in communities that recover colonization resistance more quickly. Specifically, we did note that a small subset genes previously associated with antibiotic resistance were differentially regulated following antibiotic treatment, but none of these were among the strong signals differentiating metatranscriptomes. Any conclusive findings to this end would require much more extensive investigation.

**Suggestions:**

**1) In the introduction line 55 it was mentioned “Although most classes of antibiotics have been associated with initial susceptibility to CDI, fluoroquinolones, clindamycin, and cephalosporins are linked to increased risk of recurrent or persistent infection” whereas the drugs used for the study (example line 95) are “streptomycin, cefoperazone, and clindamycin”. While cefoperazone, and clindamycin correspond to the antibiotics that have previously shown to have persistent infection, it is unclear why streptomycin was used instead of fluoroquinolones?**

This choice was made based on data in a previous publication from our laboratory (Schubert, et. al. (2015). *mBio*.), in which fluoroquinalone pretreatment did not lead to loss of colonization resistance in our model of *C. difficile* infection. We suspect that this may be due to in the microbiota across humans and mice, and therefore chose a pretreatment regime that also had resulted in continuous colonization over the observation period in addition to a cephalosporin and clindamycin as mentioned by this reviewer.

**2) Here are some examples for minor typos highlighted a) line 97 “These antibiotics were chosen for not only the ability to to reduce *C. difficile* colonization” b) line 147 “successfully differentiate microbiomes that clear infection and and”. Also, in this sentence should it be “clear infection” or “clear during infection”?**

Each of these errors have been corrected in the final version of the manuscript. We have also clarified the sentence referring to communities that are associated with *C. difficile* clearance.

**Reviewer #2 (Comments for the Author):**

**The authors present a study characterizing shifts in the microbiota, metabolites, and transcripts associated with *C. difficile* infection following treatment with various antibiotics. The experiments are well described with appropriate controls and analysis. A great deal of data is presented, which makes the results section very lengthy, and much of this section also includes rationale and analysis that would be better suited to introductory or discussion remarks. In addition, there are multiple typographical errors throughout the manuscript that should be corrected. Otherwise, this is a timely study with important information regarding the ecology behind *C. difficile* infection.**

**Specific comments:**

**lines 121-125; 183-186: Please move these sentences to the discussion.**

The authors agreed that these sentences were misplaced in the the Results section and have now moved them to the Discussion.

**line 236: Something appears to be missing from this sentence. Please clarify.**

We apologize for any confusion and agreed that some words were mistakenly omitted during the editing processes. This has new been corrected.

The authors have also made a greater effort to correct any lingering grammatical and typographical errors left in the manuscript.