AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

Effective colonization by nontoxigenic Clostridioides difficile REA strain M3 (NTCD-M3) spores following treatment with either fidaxomicin or vancomycin

Susan Sambol, Andrew Skinner, Fidel Serna-Perez, Benjamin Owen, Dale Gerding, and Stuart Johnson

Corresponding Author(s): Stuart Johnson, Edward Hines Junior VA Hospital

Review Timeline:

Submission Date:	February 2, 2023
Editorial Decision:	February 22, 2023
Revision Received:	March 3, 2023
Accepted:	March 6, 2023

Editor: Karen Carroll

Reviewer(s): Disclosure of reviewer identity is with reference to reviewer comments included in decision letter(s). The following individuals involved in review of your submission have agreed to reveal their identity: Kevin W Garey (Reviewer #1); Matthew Kyle Schnizlein (Reviewer #2)

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1128/spectrum.00517-23

February 22, 2023

Dr. Stuart Johnson
Edward Hines Junior VA Hospital
Research Service/151
Fifth Ave. and Roosevelt Rd
Hines, IL 60141

Re: Spectrum00517-23 (Effective colonization by nontoxigenic Clostridioides difficile REA strain M3 (NTCD-M3) spores following treatment with either fidaxomicin or vancomycin)

Dear Dr. Stuart Johnson:

Thank you for submitting your manuscript to Microbiology Spectrum. Your manuscript has been reviewed by 3 experts in the field and the consensus is that the work is technically sound and the manuscript is well-written, requiring only minor revisions. I am willing to accept the manuscript once all of the reviewers' comments are addressed.

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

Link Not Available

Below you will find instructions from the Microbiology Spectrum editorial office and comments generated during the review.

ASM policy requires that data be available to the public upon online posting of the article, so please verify all links to sequence records, if present, and make sure that each number retrieves the full record of the data. If a new accession number is not linked or a link is broken, provide production staff with the correct URL for the record. If the accession numbers for new data are not publicly accessible before the expected online posting of the article, publication of your article may be delayed; please contact the ASM production staff immediately with the expected release date.

The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick Author Survey.

Sincerely,

Karen Carroll

Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #1 (Comments for the Author):

NTCD colonization

This is a small hamster study to assess whether NTCD-M3 would colonize these hamsters after either vancomycin or fidaxomicin treatment. All hamsters became colonized with NTCD-M3 with both therapies. This builds upon human data to expand previous findings to now include colonization with fidaxomicin treated arms. Although perhaps not the most novel finding, this does move the development of NTCD-M3 in the right direction and agree with the authors that this supports further

development. Figure 2 is an excellent visual summary of results. I don't have any major comments but a few suggestions to improve the clarity of the manuscript.

Statistical analysis (lines 107-12). A description on the analysis plan for NTCD-M3 colonization should be provided here. I'm guessing the authors felt that 100% of hamsters would be colonized and thus, the low number of hamsters used. A power calculation here may be beneficial.
Table 1. LC-MS data may not be completely familiar to readers of MIcrobiolog Spectrum. A 1-2 line explanatory in the title may be helpful.
This study looked at colonization (Y/N) and no quantitation was performed. This should be mentioned as a limitation including why quantitation was not performed.

Reviewer #2 (Comments for the Author):

See attached Word document for detailed suggestions.

Reviewer #3 (Comments for the Author):

This is a well written and straight forward study to determine if fidaxomicin inhibits M3 colonization compared to vancomycin. This study is important because fidaxomicin use has been increasing since the first in-human studies of M3, and will likely continue to due so, and, as such, whether fidaxomicin may inhibit M3 colonization is an important question to investigate with the planned phase 3 study.

No major comments.

The minor comment is in the methods it states stool was collected out to day 30, but in the results and figures 1 and 2 stool appears to have been collected out to day 43. Please update the methods.

Staff Comments:

Preparing Revision Guidelines
To submit your modified manuscript, log onto the eJP submission site at https://spectrum.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

• Point-by-point responses to the issues raised by the reviewers in a file named "Response to Reviewers," NOT IN YOUR COVER LETTER.
• Upload a compare copy of the manuscript (without figures) as a "Marked-Up Manuscript" file.
• Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
• Manuscript: A .DOC version of the revised manuscript
• Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at https://journals.asm.org/journal/Spectrum/submission-review-process. **Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript.** "

Please return the manuscript within 60 days; if you cannot complete the modification within this time period, please contact me. If you do not wish to modify the manuscript and prefer to submit it to another journal, please notify me of your decision immediately so that the manuscript may be formally withdrawn from consideration by Microbiology Spectrum.

If your manuscript is accepted for publication, you will be contacted separately about payment when the proofs are issued; please follow the instructions in that e-mail. Arrangements for payment must be made before your article is published. For a complete list of **Publication Fees**, including supplemental material costs, please visit our website.

Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

Thank you for submitting your paper to Microbiology Spectrum.

Summary

Colonization with a non-toxigenic *C. difficile* strain following antibiotic treatment has been shown to lower risk of recurrence in humans and hamsters. While colonization of *C. difficile* strain M3 (NTCD-M3) following vancomycin treatment has been demonstrated, colonization following fidaxomicin has not, despite it being the current clinically-preferred antibiotic. The authors sought to establish that strain NTCD-M3 colonizes similarly in both antibiotic courses (i.e., fidaxomicin in addition to the previously studied vancomycin) using a hamster model of *C. difficile* colonization that mirrors the authors' phase 2 clinical trial conditions (Gerding et al., *JAMA*, 2015). They found that hamsters in each group became colonized at similar time periods following the cessation of antibiotics, which tended to associate with gradually decreasing fecal antibiotic concentrations. The authors suggest that this provides a foundation for future clinical trials investigating the treatment efficacy of colonizing with non-toxigenic *C. difficile* strain NTCD-M3. This is a succinct manuscript bringing novel information to the scientific community regarding possible *C. difficile* preventative measures. It is well-controlled and generally draws conclusions that can be readily gathered from the data presented.

Specific comments

Major points:

1. None

Minor points:

1. L52: if trying to indicate hamsters were housed individually, state "individually housed in polycarbonate cages…" The current reading suggests that several hamsters where group-housed in a couple individual cages.
2. L80: consider adding something along the lines of, "in the absence of study drug-mediated microbiota perturbations" to more clearly state the purpose of the control.
3. L130: As a non-clinician, the broader context of the MICs as stated was not readily apparent (i.e., are these normal resistance levels?). Consider adding, "…, which are similar to previously reported MICs for other *C. difficile* strains" and citing Gargis et al. (10.1093/cid/ciac817), Thorpe et al. (10.1128/AAC.00391-19) and re-citing Goldstein et al., *Clin Infec Dis*, 2012.
4. L132-142/Figure 2: Does the variability in hamster colonization at Day 9 (3rd M3 gavage) associate with fecal antibiotic concentrations, particularly the variability in OP-1118 levels? If so, adding a statement to that effect would strengthen the claim at L160 about colonization being associated with gradual decreases in antibiotic concentrations. Otherwise, consider adding a statement indicating the lack of association and that the factors involved in partial colonization were extraneous to this study.
5. L165: "This delay correlated with…" The authors use "correlate" without offering any statistical backing for their statement in the results. Please use an appropriate statistical test or alternative wording as in L159 ("appears to be associated").
6. Other: If M3 colonization levels were titered (rather than collecting just presence/absence data), this reviewer would very much appreciate those data being included in a supplement. While they are not essential for the manuscript's conclusions, they would be useful to the scientific community.

Response to Reviewers

Reviewer #1:
Statistical analysis (lines 107-12). A description on the analysis plan for NTCD-M3 colonization should be provided here. I'm guessing the authors felt that 100% of hamsters would be colonized and thus, the low number of hamsters used. A power calculation here may be beneficial.

Response: We have extensive experience with non-toxigenic *C. difficile* colonization in hamsters. NTCD-M3 historically colonizes 90- 100% of hamsters after antibiotic treatment [manuscript references: 4-8]. Using groups of 10 hamsters allows for the detection of significant declines in colonization of 50% from that of the expected colonization rate (P=0.03) and allows comparison with other data collected historically in our lab. We have now included a brief summary in the statistical methods section.

Table 1. LC-MS data may not be completely familiar to readers of MIcrobiolog(y) Spectrum. A 1-2 line explanatory in the title may be helpful.

Response: We have now included additional explanation of the data in a footnote to the table.

This study looked at colonization (Y/N) and no quantitation was performed. This should be mentioned as a limitation including why quantitation was not performed.

Response: Semi-quantitative cultures were performed (1+ to 4+) but the transition from negative to high levels (3+ to 4+) occurred rapidly and was not captured within our sampling time periods (hamster pellets were collected daily from the cages). Hamsters were typically culture negative one day and 3+ or 4+ the next day. We have included the raw data for semi-quantitative cultures as supplemental files and we have now modified our methodology and results section to include these semi-quantitative culture results.

Reviewer #2:
If trying to indicate hamsters were housed individually, state “individually housed in polycarbonate cages…” The current reading suggests that several hamsters where group-housed in a couple individual cages.

Response: We have now changed the wording to clarify that hamsters were individually housed rather than group housed.

Consider adding something along the lines of, “in the absence of study drug-mediated microbiota perturbations” to more clearly state the purpose of the control.

Response: We have now added the suggested wording to clarify the purpose of the 2 control hamsters in each experimental group that did not receive the study antibiotics.

As a non-clinician, the broader context of the MICs as stated was not readily apparent (i.e., are these normal resistance levels?). Consider adding, "…, which are similar to previously reported MICs for other *C. difficile* strains" and citing Gargis et al. (10.1093/cid/ciac817), Thorpe et al. (10.1128/AAC.00391-19) and re-citing Goldstein et al., *Clin Infec Dis*, 2012.

Response: We have now added these additional references in the results section after giving the MIC results for the study antibiotics towards NTCD-M3 to put these results in context of previously reported data as suggested.

Does the variability in hamster colonization at Day 9 (3rd M3 gavage) associate with fecal antibiotic concentrations, particularly the variability in OP-1118 levels? If so, adding a statement to that effect would strengthen the claim (at L160) about colonization being associated with gradual decreases in antibiotic concentrations. Otherwise, consider adding a statement indicating the lack of association and that the factors involved in partial colonization were extraneous to this study.

Response: We reviewed the fecal concentrations of OP-1118 for the hamsters who were colonized and not yet colonized on day 9. The median OP-1118 value was lower in colonized hamsters at 5.32 (IQR: 2.43 – 6.54) µg/gm feces when compared to the non-colonized hamsters with a median value of 7.33 (IQR: 6.16 – 20.98) µg/gm feces (p=0.14). While not significant, the trend supports our statement that colonization was associated with gradual decreases in antibiotic concentrations. The results and discussion are modified to show these data.

"This delay correlated with…" The authors use "correlate" without offering any statistical backing for their statement in the results. Please use an appropriate statistical test or alternative wording (as in L159) ("appears to be associated").

Response: We have now changed the wording in the discussion to 'appeared to be associated' as suggested by the reviewer.

If M3 colonization levels were titered (rather than collecting just presence/absence data), this reviewer would very much appreciate those data being included in a supplement. While they are not essential for the manuscript's conclusions, they would be useful to the scientific community.

Response: As mentioned in the response to reviewer #1, semi-quantitative cultures were performed, but colonization of the hamsters occurred quickly and the transition from negative to 4+ culture positive was not picked up in our sampling frequency. We have updated the methods and results sections to include the semi-quantitative culture data. We have also included the raw data files for the culture results supplemental files.

Reviewer #3:
In the methods it states stool was collected out to day 30, but in the results and figures 1 and 2 stool appears to have been collected out to day 43. Please update the methods

Response: We have now clarified that fecal pellets were collected until 30 days after the end of spore administration (day 43).

March 6, 2023

Dr. Stuart Johnson
Edward Hines Junior VA Hospital
Research Service/151
Fifth Ave. and Roosevelt Rd
Hines, IL 60141

Re: Spectrum00517-23R1 (Effective colonization by nontoxigenic Clostridioides difficile REA strain M3 (NTCD-M3) spores following treatment with either fidaxomicin or vancomycin)

Dear Dr. Stuart Johnson:

Thank you for addressing the reviewers' comments and for including the supplemental material. Your manuscript has been accepted, and I am forwarding it to the ASM Journals Department for publication. You will be notified when your proofs are ready to be viewed.

The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick Author Survey.

Publication Fees: We have partnered with Copyright Clearance Center to collect author charges. You will soon receive a message from no-reply@copyright.com with further instructions. For questions related to paying charges through RightsLink, please contact Copyright Clearance Center by email at ASM_Support@copyright.com or toll free at +1.877.622.5543. Hours of operation: 24 hours per day, 7 days per week. Copyright Clearance Center makes every attempt to respond to all emails within 24 hours. For a complete list of **Publication Fees**, including supplemental material costs, please visit ourwebsite.

ASM policy requires that data be available to the public upon online posting of the article, so please verify all links to sequence records, if present, and make sure that each number retrieves the full record of the data. If a new accession number is not linked or a link is broken, provide production staff with the correct URL for the record. If the accession numbers for new data are not publicly accessible before the expected online posting of the article, publication of your article may be delayed; please contact the ASM production staff immediately with the expected release date.

Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

Thank you for submitting your paper to Spectrum.

Sincerely,

Karen Carroll
Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org