Author’s response to reviews

Title: E. coli diversity: low in colorectal cancer

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Dear Editor,

Many thanks to you for your work on our manuscript (MGNM-D-19-00140R1). We have made the corrections accordingly as detailed below. Here we submit a cover letter with point-by-point responses to the comments, a revised manuscript with the changes marked, and a clean copy of the updated manuscript.

Best regards,

Shu-Lin Liu, MD, PhD

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Editor Comments:

Please ensure that the comments raised by reviewer 1 below are addressed in full in this round of revisions.

Reviewer reports:

Thomas H. Hampton, Ph.D (Reviewer 1): The authors read my request for statistical clarity but have declined to provide it. Figure 2B now includes dot plots of the number of strains identified in each group, as well as text suggesting a statistical test was performed. The authors do not specify the test they used in the methods or elsewhere, but I am guessing they used pairwise t tests. The t test is a true workhorse, but not really appropriate for count data. I see better from the...
figure what they are driving at: the CRC samples were notably lower than the seniors they measured. It looks as though the number of distinct species increases with age. Therefore, the correct design is to compare CRC patients to age matched controls and use an appropriate statistic for the comparison. The fact that children (in addition to CRC patients) have relatively few E. coli strains is not the focus of this study. This remains interesting, but in my opinion the authors should talk to a statistician before attempting to resubmit this work.

Response: As suggested by the reviewer, we provided the statistical clarity in Methods (Lines 146-149 of the revised manuscript). We also added statistical test results in Figure 2B legend.

Statistical analysis (Lines 146-149 of the revised manuscript)

Statistical analysis was conducted by using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism statistical software; as the data were not normally distributed, Wilcoxon rank-sum test was used.

We also modified Figure 2B legend as follows.

Figure 2. Diversity of E. coli in different age and health status groups. A, Levels of E. coli diversity in the individual groups. The diversity is illustrated by percentages of participants in a group that have one, two or more genomic types among the E. coli strains analyzed. B, Statistical comparisons of E. coli diversity among the four groups. ***: p \textless 0.001 (Children vs Students, p-value = 3.291e-09; Children vs Seniors, p-value = 3.104e-10; Students vs Seniors, p-value = 7.226e-06; Seniors vs CRC patients, p-value = 6.83e-06; Students vs CRC patients, p-value = 0.0039; Children vs CRC patients, p-value = 0.8847.). Note that most CRC patients had only one genotype and most senior individuals had five or more genotypes.

Although the number of distinct species (E. coli genotypes) seems to increase with age, our focus in this study is on the low genetic diversity of E. coli genotypes in CRC patients. Ideally, the correct design is to compare CRC patients to age matched controls, but it is not easy to have sufficient number of CRC patients among seniors of 90 years old and up; we agree that this is a good suggestion and will be a goal of our next step of this series of studies.

Ana Lucia Fachin (Reviewer 2): The authors response properly the all questions. I considered the manuscript approved for publication.

Response: We highly appreciate the time and work that both reviewers and the editors put into the manuscript, especially all comments and suggestions provided, which have greatly helped us to improve the readability of this piece of work.