Dear Editors,

We would like to express our sincere gratitude to the reviewers for their valuable feedback and constructive criticism on our manuscript. We appreciate their time and effort in reviewing our work and their insightful comments have greatly improved the quality of our research.

Here are our responses to the reviewers:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: In this review, the authors summarize the life cycle and toxicity Clostridioides difficile, diagnostic approaches, including microRNAs. The author wants to emphasize the importance of microRNA, but there is not much about microRNA in this article. The main content is to introduce CDI, including its pathogen characteristics, diagnostic methods, etc. Therefore, if the author wants to emphasize microRNA, as in the title, it is necessary to add more information about microRNA, including specific molecular mechanism, diagnostic methods, sensitivity, advantages and disadvantages, etc. Alternatively, the author can modify the title to: The overview of current detection methods in Clostridium difficile infection.

Thank you for your valuable considerations and comments; we do agree with your statements and increased the information about miRNAs and additionally modified the title "reducing" microRNA impact as follows: "The overview of current detection methods and microRNA potential in *Clostridium Difficile* infection screening".

Here we report the changes and addition made to the manuscript in order to make it easier to read:

"A portion of the miRNA, the seed sequence, which is two to eight nucleotides long, pairs with a specific sequence on the target mRNA and is referred to as miRNA response elements (MREs) that result in translational repression and degradation of the target mRNA due to the binding of microRNAs in the 3' untranslated region (3'UTR). miRNAs primarily repress genes by inhibiting protein synthesis, preventing elongation and

ribosome decline, and disrupting mRNAs through the processes of demethylation and decap, resulting in their degradation. MicroRNA could potentially act by this molecular mechanism in every step of CDI, inhibiting specific transcripts or inflammatory molecules transcription and therefore influencing the pathology grade. Those biomarkers' unphysiological unbalance can be measured and exploited for diagnosis."

“In particular, microRNA are easily detectable (through sequencing, RT-qPCR, etc.) in body fluids such as saliva, blood, and even fecal material, and their levels correlate with target transcript alterations or non-physiological events[117]. These biomarkers are contributing more and more to the establishment of less invasive "liquid biopsy," which is important and appealing for patients' compliance compared to normal, invasive biopsies and analyses that take a long time for results. Although, currently, it cannot fully substitute canonical diagnosis methods in most cases, it will undoubtedly happen in the near future.”

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: This review provides an overview of the CDI process and the relationship to different techniques and technological approaches. Subsequently, it offers a detailed description of the reviewed techniques, as well as the scope of each of them. This review also provides detailed information on the publications that exist in this field of interest and the technical scope of these publications. It is also possible to find information on diagnostic strategies and the importance of miRNAs. The expression of miRNAs has become increasingly important as new biomarkers for assessment. The different signature profiles obtained through the differential expression of these small non-coding RNAs are essential for early diagnosis and treatment prediction disease response and management, which also includes cancer and infections. In the manuscript review process and to ensure the integrity and quality of the process, I have considered two questions: (1) Is the manuscript important/innovative and why? and (2) Is the manuscript well organized and presented in a concise and coherent manner? My answer to both questions is affirmative and I consider that it meets the quality standards for its publication. Revised the manuscript in its entirety, and additional information. I agree with other previous comments and already added by the authors (possibly from previous reviews). The order of the revision argument is correct. The references are those that reflect the

state of the art in the bibliography. I would like the authors to indicate how the figures have been made (they seem to be of very good quality). I am commenting on this, because if the source is Biorender, it is mandatory to cite the origin of the figures. Lastly, in Figure 3, I would substitute the “sample jar” on the left for a more appropriate one. and I would correct the term FACAL by FECAL.

Thank you for your deep and concise analysis of our work. As you noticed, we've already amended and revised the whole manuscript according to the reviewers' comments and we are pleased you find it ready for publication. We corrected the typo and added the acknowledgement for the Biorender-made figure. We do apologize since we missed this in the first place.

Moreover, Dr. Benjamin Dickins believed that his contribution was not significant enough to merit authorship, and as a result, we have excluded him as a co-author. Instead, we express our gratitude towards him in the acknowledgements.

We deeply thank the Reviewers, the Editors and the Journal for this opportunity.
Sincerely,

Payton Yau
Research Associate / Lecturer
Soctland's Rural College / Nottingham Trent University

Marco Bocchetti
Post-Doctoral Researcher
University of Campania “Luigi Vanvitelli” / Biogem Scarl