

Exploring the Pathogenesis of *Clostridium difficile* Infection Based on Weighted Gene Co-expression Network Analysis

Yankun Li *

School of Hunan Normal University, Changsha Hunan, 410081, China

* Corresponding author: Yankun Li

Abstract: *Clostridioides difficile*, a Gram-positive, spore-forming, anaerobic bacterium, is a leading cause of antibiotic-associated diarrhea and colitis. Its infections, often linked to antibiotic use in healthcare settings, range from mild diarrhea to pseudomembranous colitis. This study explores the pathogenic and toxin action mechanisms of *Clostridioides difficile*, particularly its toxins TcdA and TcdB, which disrupt host cells through glycosyltransferase activity, affecting cell structure and intestinal integrity, while also triggering inflammation and immune responses. Treatment strategies for *Clostridioides difficile* infection continue to evolve, encompassing antibiotics, microbiota replacement, monoclonal antibodies, and emerging therapies. The study employs Weighted Gene Co-expression Network Analysis (WGCNA) to analyze the GSE29008 dataset, categorizing samples into normal, toxin A, and toxin B groups. Notably, the 'midnightblue' module shows a strong positive correlation with toxin B, indicating its significant role in severe inflammation and tissue damage, emphasizing the importance of understanding the toxin action mechanisms for developing effective treatments and public health policies.

Keywords: WGCNA; *Clostridioides Difficile*; Toxins TcdA and TcdB; Colitis.

1. Introduction

Clostridioides difficile is a common cause of antibiotic-associated diarrhea and colitis. This anaerobic bacterium produces toxins that lead to various symptoms, from mild diarrhea to pseudomembranous colitis. Its infections are closely linked to antibiotic use, particularly in healthcare settings. Treatment approaches, including sensitive diagnostics and therapies like antibiotics, microbiota replacement, and monoclonal antibodies, are evolving. The toxins TcdA and TcdB disrupt host cells through glycosyltransferase activity, affecting cell structure and intestinal integrity. These toxins also trigger inflammation and immune responses, potentially leading to acute and chronic inflammation. In this study, I utilized the bioinformatics method known as WGCNA to analyze the GSE29008 dataset.

2. The Pathogenic Mechanism and Toxin Action Mechanism of *Clostridium Difficile*

Clostridioides difficile (formerly known as *Clostridium difficile*) is a Gram-positive, spore-forming, anaerobic bacterium, considered to be the most common cause of antibiotic-associated infectious diarrhea and colitis. This bacterium can produce toxins, leading to a range of disease symptoms, from mild diarrhea to pseudomembranous colitis.[1]

Clostridioides difficile infection is often closely associated with the use of antibiotics. The use of antibiotics disrupts the balance of normal intestinal flora, providing an opportunity for the proliferation and colonization of *Clostridioides difficile*. Infections are widespread globally, especially in hospitals and long-term care facilities. The high recurrence rate and treatment challenges make *Clostridioides difficile*

infection a significant clinical concern.

Treatment methods for *Clostridioides difficile* infection continue to advance, including more sensitive and specific diagnostic tools, as well as various treatment options such as antibiotic therapy, microbiota replacement therapy, and monoclonal antibody use. Future treatment strategies may involve combinations of multiple therapies to provide more effective and robust management.

Regarding the two main toxins of *Clostridioides difficile*, TcdA and TcdB, their effects on host cells are achieved through glycosyltransferase activity that disrupts the cell's cytoskeleton, primarily by inactivating Rho GTPases, leading to changes in cell morphology and loss of intestinal integrity. Both toxins have similar domain structures, including glycosyltransferase domain, autocleavage domain, translocation domain, and CROPS domain. TcdA and TcdB bind to host cell surface receptors and enter cells through receptor-mediated endocytosis. These toxins are released inside the cells and cause cell rounding and eventual cell death by disrupting the host cell's cytoskeleton and tight junctions.[2]

Furthermore, *Clostridioides difficile* toxins can stimulate the host to produce an inflammatory response. Damaged epithelial cells release cytokines and chemokines, recruiting immune cells such as neutrophils to the infected tissue. The effects of toxins on monocytes and macrophages may promote the production of IL-1 β and cell pyroptosis, exacerbating inflammation and damage within the tissue. Overall, these processes constitute the pathological basis of *Clostridioides difficile* infection, with the impact on the immune system potentially leading to acute and chronic inflammatory responses. The immune response triggered by toxins primarily manifests in their effects on immune cells, such as the release of pro-inflammatory cytokines, which can result in a broader immune response and tissue damage. These reactions collectively affect the host's immune system,

potentially leading to the occurrence of acute and chronic inflammatory responses.

3. Methods of Infection, Treatment Outlook, and Challenges for Public Health Policy in Clostridium difficile Infections

Treatment methods for Clostridioides difficile infection continue to evolve and innovate. Currently, the primary treatment involves the use of specific antibiotics, although this approach carries some risks, it has been proven effective in treating recurrent C. difficile infections. Additionally, research indicates that neutralizing antibodies against C. difficile toxins TcdA and TcdB can effectively inhibit their toxicity. For example, antibodies targeting TcdB can prevent the development of severe gastrointestinal and systemic diseases. Antibodies developed using phage display technology have demonstrated effective neutralization of both TcdA and TcdB. Furthermore, humanized monoclonal antibodies have shown effectiveness in vitro neutralizing various clinical strains and in animal models.[3]

Future research directions include a deeper exploration of the mechanisms of action of TcdA and TcdB toxins. For instance, studies using nanobodies targeting TcdA and TcdB have revealed unexpected neutralization epitopes. Meanwhile, the development of novel treatment strategies, such as small-molecule drugs targeting specific toxin functional regions, has shown potential in inhibiting TcdB activity. Additionally, vaccine development is an important direction, with DNA-based vaccines shown to generate protective immunity against TcdA and TcdB, and oral immunization demonstrating protective effects against C. difficile infection.[4]

Antibiotic resistance is a major challenge in C. difficile infection research. With increasing antibiotic use, the problem of antibiotic resistance in C. difficile has become more severe. For example, an epidemiological surveillance study in Chongqing, China, identified the emergence of multidrug-resistant strains, highlighting the seriousness of this issue. The increase in community-acquired C. difficile infections has also become a public health threat, especially in populations traditionally considered low-risk.

To address these challenges, public health policies emphasize the importance of hospital infection control and

the prudent use of antibiotics. Ensuring the timely identification and treatment of symptomatic C. difficile infection patients is crucial, as most patients respond rapidly to antimicrobial therapy. The best strategy for preventing C. difficile infections is the implementation of effective infection control measures and the rational use of antibiotics.[5]

4. WGCNA Analysis Conducted on the GSE29008 Dataset

In this study, I employed the 'WGCNA' package in R software to analyze the GSE29008 dataset from the GEO database. Using the 'goodSamplesGenes' method, I excluded obviously abnormal samples and genes. During the construction of the scale-free co-expression network, I first calculated the Pearson correlation matrix between pairs of genes. Subsequently, I used the average linkage method to build a weighted adjacency matrix.

In the study, I chose a value of $\beta = 18$. β is a soft-thresholding parameter that strengthens strong correlations between genes and penalizes weak correlations. I also utilized the Topological Overlap Matrix (TOM) to measure the connectivity of the gene network. TOM is calculated based on adjacency and represents the sum of adjacency between a gene and all other genes in the network. It can also be used to compute dissimilarity(1-TOM). I performed hierarchical clustering based on TOM dissimilarity, grouping genes with similar expression profiles into different gene modules. The minimum module size for the gene dendrogram was set to 30 with a sensitivity of 3. In subsequent gene module analysis, I calculated the differentiability of module characteristic genes and merged modules with a distance less than 0.25. We also selected a cutting line to generate a module dendrogram, resulting in a total of 33 co-expression modules. The gray module contained genes that couldn't be assigned to any other module. Finally, by calculating the correlation between module eigengenes and gene expression, we obtained Module Membership.

In the study, I used WGCNA to primarily categorize samples into three groups: the normal group, toxin A group, and toxin B group. Through the application of the Weighted Gene Co-expression Network Analysis(WGCNA) method, I performed comprehensive analysis of these three variables: normal group, toxin A group, and toxin B group.

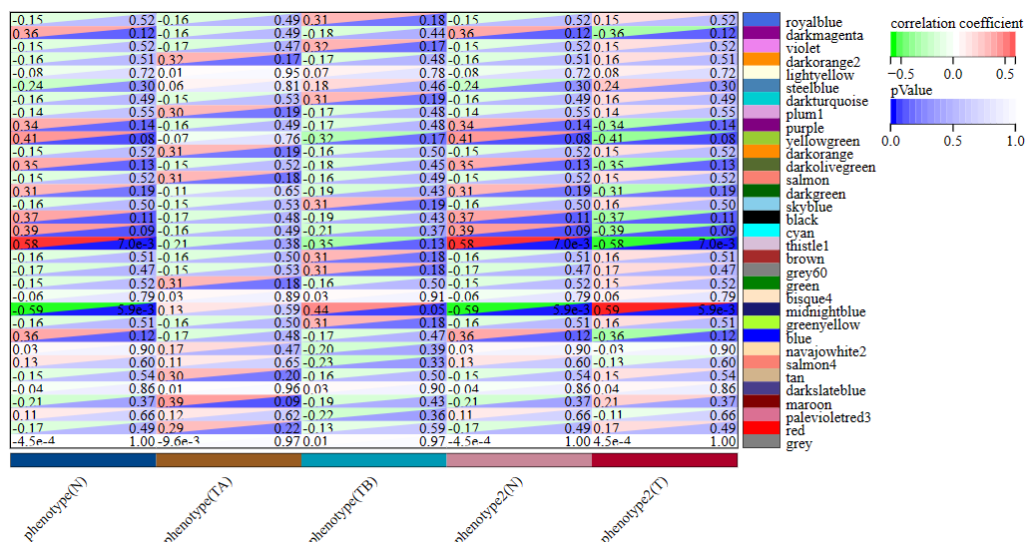


Figure 1. Weighted Co-expression Network Analysis of the GSE29008 dataset. Cluster analysis was performed on all samples, with a module merge height of 0.25, a soft threshold β of 18, an independence value of 0.94, resulting in the construction of 33 modules

In the process of constructing a scale-free network, I chose a soft threshold β of 18 and an independence of 0.94 to establish the topological matrix. We clustered differentially expressed genes with similar expression patterns into 33 different modules. Of particular note is the 'midnightblue' module, which exhibited a strong positive correlation with toxin B (correlation coefficient = 0.44, p-value = 0.05) and a strong negative correlation with the control group (correlation coefficient = -0.59, p-value = $5.9e-3$). This demonstrates that TcdB in *Clostridium difficile* toxin exhibits stronger cytotoxicity compared to TcdA, and is a significant contributor to severe inflammation and tissue damage.

5. Conclusion

In this study, through bioinformatics analysis of the GSE29008 dataset using Weighted Gene Co-expression Network Analysis (WGCNA), I have identified the significant role of the toxin TcdB in *Clostridioides difficile* infection. Specifically, I observed a strong positive correlation between the "midnightblue" module in WGCNA and TcdB, indicating that TcdB plays a more crucial role in severe inflammation and tissue damage compared to TcdA. This finding not only enhances our understanding of *Clostridioides difficile* infections but also provides deeper insights into the mechanisms of action of *Clostridioides difficile* toxins. This discovery holds valuable implications for the treatment and prevention of *Clostridioides difficile*-

related diseases.

References

- [1] Kordus SL, Thomas AK, Lacy DB. *Clostridioides difficile* toxins: mechanisms of action and antitoxin therapeutics. *Nat Rev Microbiol*. 2022 May;20(5):285-298. doi: 10.1038/s41579-021-00660-2. Epub 2021 Nov 26. PMID: 34837014; PMCID: PMC9018519.
- [2] Del Prete R, Ronga L, Addati G, Magrone R, Abbasciano A, Decimo M, Miragliotta G. *Clostridium difficile*. A review on an emerging infection. *Clin Ter*. 2019 Jan-Feb;170(1): e41-e47. doi: 10.7417/CT.2019.2106. PMID: 30789196.
- [3] Giles J, Roberts A. *Clostridioides difficile*: Current overview and future perspectives. *Adv Protein Chem Struct Biol*. 2022; 129:215-245. doi: 10.1016/bs.apcsb.2021.11.003. Epub 2022 Jan 7. PMID: 35305720.
- [4] Colleen R Kelly, Jessica R Allegretti, Review Article: Gastroenterology and *Clostridium difficile* Infection: Past, Present, and Future, *Clinical Infectious Diseases*, Volume 77, Issue Supplement 6, 1 December 2023, Pages S463–S470, <https://doi.org/10.1093/cid/ciad644>.
- [5] Czepiel J, Drózdź M, Pituch H, Kuijper EJ, Perucki W, Mielimonka A, Goldman S, Wultańska D, Garlicki A, Biesiada G. *Clostridium difficile* infection: review. *Eur J Clin Microbiol Infect Dis*. 2019 Jul;38(7):1211-1221. doi: 10.1007/s10096-019-03539-6. Epub 2019 Apr 3. PMID: 30945014; PMCID: PMC6570665.