The Prevalence of *Clostridium difficile* in the Wisconsin Area and the Role of Dietary Fiber on Colonization

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**Abstract**

*Clostridium difficile* is an opportunistic pathogen. Since antibiotics alter a human’s microbiota, hospitalized patients are more likely to become infected with *C. difficile*. Studies have shown that a high-fiber diet can reduce the prevalence of multi-drug resistant organisms (MDROs). These findings suggest that a high-fiber diet could reduce the colonization of *C. difficile*. Throughout this study, the influence of dietary on the prevalence rate of *C. difficile* will be discovered within the Wisconsin area. Surveys administered by the Survey Health of Wisconsin (SHOW) will be used to determine the fiber intake of participants. The presence of *C. difficile* will be discovered by isolating colonies with *C. difficile* morphology, from stool samples, and then performing polymerase chain reactions (PCR).

**Introduction**

*Clostridium difficile* is the most common cause of infectious diarrhea in hospitals, accounting for 20-30% of all cases. Antibiotics only increase one’s risk of acquiring the disease because they disrupt the normal gut microflora, allowing *C. difficile* to grow and produce toxins.1

Some studies have found that diet can influence the colonization of these harmful bacteria. A high-fiber diet has been proven to be associated with the reduced prevalence of multi-drug resistant organisms or MDROs, including methicillin-resistant *Staphylococcus aureus* (MRSA),
vancomycin-resistant enterococci (VRE), and fluoroquinolone-resistant Gram-negative bacteria (FQRGNB). Fiber likely played an important role in reducing MDROs because it increases gut microbial diversity which can lead to increased immune function and more competition for MDROs. Since fiber was successfully able to influence the colonization of MDROs, it was plausible it may do the same for the colonization of Clostridium difficile.

The aim of this study was to determine the prevalence rate of C. difficile in the Wisconsin area and discover whether dietary fiber influences the colonization of C. difficile in the human gut microbiota.

**Methods**

**Patient Population/ Sample Collection**

The Survey Health of Wisconsin, also known as SHOW, is a Madison based research infrastructure that gathers annual health surveys on Wisconsin residents. In order to determine the health determinants and outcomes of dietary fiber, SHOW surveyed 6,000 individuals on their fiber intake. For this project, 559 random stool samples were used from these 6,000 participants to help evaluate the correlation between dietary fiber and the colonization of Clostridium difficile in the human gut. Of the people who participated in SHOW’s study, majority were Caucasian between the ages of 50-69. There was also about an equal number of females and males who participated and about an equal distribution of education.

**Microbial Work-up**
The 5ml stool samples were first taken from the -80°C and then 100-200 mg of *C. difficile* was added to 1 ml of *Clostridium difficile* Brucella Broth (CDBB). The CDBB has antibiotics and germinates making it selective for *C. difficile*. These liquid cultures were then incubated anaerobically at 37°C for 24 hours.

After they were allotted enough time to incubate, 50µl of each liquid culture was streaked onto plates of *Clostridium difficile Brucella Agar* (CDBA). The CDBA also has antibiotics and germinates making it selective and differential for *C. difficile*. These plates were then allowed 48 hours to incubate anaerobically at 37°C.

After 48 hours, the CDBA plates were examined for colonies that resembled *C. difficile*. These colonies looked ground, glass, irregular edged, and yellow. The colonies that resembled *C. difficile* were then streaked onto blood agar plates and allowed 24 hours to incubate anaerobically at 37°C.

After these plates were allotted 24 hours to incubate, isolates were taken from the pre-reduced BAPs and gram stains and catalase tests were performed. If the isolate was catalase-negative and a gram-positive rod with spores, it was then stocked in a tryptic soy broth (TSB) with 20% glycerol and stored it in the -80°C freezer.

**Molecular Work-up**

The isolates that were confirmed catalase-negative and gram-positive rods were then added to a crude lysate for polymerase chain reaction (PCR) testing. PCR allowed me to confirm if the
suspected isolates were *C. difficile*. The in-house PCR had four target genes: an enolase gene (housekeeping gene), toxin A, toxin B, and binary toxin CDTB.

**Results**

For the results, the participant’s predicted fiber consumption data was split it into four equal quartiles shown by the four different bars on the graph. The predicted fiber consumption was an estimate of dietary fiber for each participant based off their food frequency questionnaires. In each quartile, the percentage of positive cases was calculated. In the first quartile, 0 to 10.46 grams of fiber per day, 9.3% of the samples tested positive for *C. difficile*. In the second quartile, 4% of the samples tested positive. In the third quartile, 8.7% of the samples tested positive and in the fourth quartile 4% of samples tested positive for *C. difficile*. 
Of all the stools that tested positive for *C. difficile*, 71% were toxigenic and 29% were non-toxigenic. As dietary fiber increased, the percentage of toxigenic cases went down. In the fourth quartile, there was an equal number of toxigenic to non-toxigenic positive *C. difficile* cases. In this limited study it was concluded that dietary fiber does not influence the colonization of *Clostridium difficile*. This is because as the amount of dietary fiber increased, the amount cases that tested positive for *C. difficile* did not decrease. These results could have been influenced by the large number of participants that were consuming under the recommended amount of dietary fiber.
This graph shows how many samples were in each group of predicted fiber consumption. The red line shows the recommended daily fiber intake according to Mayo Clinic, which is around 30 grams of fiber per day. As shown by the graph, most of the participants in this study were consuming below the recommended amount of dietary fiber.

**Conclusion**

**Strengths/ Limitations**

One limitation of this study was the population was made up of mostly Caucasian, Wisconsin, residents. A more diverse group of samples and microbiomes could have potentially responded differently to dietary fiber. Participants were also self-reporting the amount dietary fiber they were consuming which could have allowed for an inaccurate account of their actual dietary fiber intake. The analysis of the data was simple. If more variables had been considered such as the participant’s age, gender, or concurrent health problems, different results may have been seen. Freezing the stool samples in the -80°C freezer could have killed a great amount of viable *C. difficile*, meaning fewer participants would have tested positive. Transient colonization could have also affected the results. *C. difficile* is not permanent, it comes and goes within a person’s life. The one stool sample that each participant provided may not have accurately been informative on whether the participant was vulnerable to *C. difficile* or not. Some strengths of this study were the large stool sample size and the samples were taken from a healthy population. The larger sample size allowed for a greater variety of participants to evaluate the effects of dietary fiber on. Because the samples were taken from a healthy population, the prevalence rate of *C. difficile* could be determined of the general population.
Future Directions

For the future, stool samples should be collected from a greater variety of geographic regions and ethnicities in order to determine how dietary fiber affects the colonization of *C. difficile* in a larger mix of people. More than one stool sample should also be collected and tested per patient to more accurately conclude whether the patients are vulnerable to *C. difficile*. For the future, a more accurate tracker of each participant’s dietary fiber consumption would also be beneficial, so that the participants are not just estimating their dietary fiber intake. It should also be enforced that each participant consumes at or above the recommended amount of dietary fiber, 30 grams per day, so that it can be concluded whether fiber actually does play a role in the colonization of *C. difficile* or not. In the future, a control group should also be added who are all consuming the same amount of dietary fiber so that it is easier to compare the effectiveness of dietary fiber on *C. difficile* colonization.
References


