Supernatant Synbiotic Formula for the management of CDAD

Clostridiun difficile – associated disease (CDAD) is relatively new disease, which was first described in the 1980s (1). *C. difficile* is the most common infectious cause of acute diarrhea in some health-care facilities. More than 90% of the infections occur after or during antimicrobial therapy (9). CDAD rates and severity are increasing possibly due to the new strains with different, more severe pathogenic properties (2).

Leading Causes and Factors for CDAD

There are several factors involved in consistently changing pathogenesis of *C. difficile (3).* The first factor is exposure to antibiotics. They are given to destroy the pathogen, but they also disrupt the protective microflora in the gastrointestinal tract. When the protective barrier is disrupted the human organism may become susceptible to pathogenic bacteria including *C. difficile.*

The second factor is a direct or indirect exposure to *C. difficile* that usually occurs in hospitals (4). Hospital patients that are carriers may contaminate the hospital environment with vegetative cells or resistant spores of *C. difficile.* The spores can stay in the hospital environment for months and it is very difficult to eliminate them. When this microorganism colonizes the human intestines it damages the enterocytes due to the release of three types of toxins: A, B and binary toxin (5). Usually *C. difficile* reproduces in the intestinal crypts and releases the toxins that cause severe inflammation. Intestinal mucous is damaged leading to the formation of pseudo membranes, injury of the colonic epithelial cells. Severe form of watery diarrhea and pain are pathognomonic clinical signs of CDAD (2).

The third factor is directly related to the invaded host-organism (6), its ability to resist or eliminate the infection. The ability of the host's immune system to produce protective antibodies against the toxins of *C. difficile* plays very important role in reducing the severity of disease and avoiding later recurrences. However, the antibodies alone do not restore the normal function of the GI tracks, and the ability to resist colonization from further exposure of spores in the environment, or to affect positively other host risk factors (7, 8) such as:

- Age greater than 65 years

- Severe underlying illness
- Naso-gastric intubations
- Anti-ulcer medications
- Longer hospital stay

Gastrointestinal tract of the neonates can also be colonized by toxigenic strains of *C. difficile,* but paradoxically they do not develop symptomatic disease (10). This is probably because of lack of receptors for toxin A in their immature enterocytes (11).

In conclusion, it is clear that there are multiple factors playing a role in the transmission of *C. difficile* and development of CDAD. In addition to the good hygiene practices in the health-care facilities, the pathogenic properties of the *C. difficile* strains and the ability of the host's immune system to resist and cope with the disease are the most important to focus upon and combat the infection successfully.

Clinical Treatments of the CDAD

- Antibiotics
- Absorbents
- Ion-exchange resins
- Immune products (antibodies)
- Faecal enemas and bowel irrigation
- Probiotics

Probiotics as Adjunctive Clinical Therapy of CDAD

Probiotics are live or inactive cells of mono- or multi-species and strains of lactic acid bacteria mainly *Lactobacilli and Bifidobacteria*. The specific properties of the microorganisms included in the probiotic formula are postulated to: (12)

- enhance the re-establishment of the normal intestinal microflora
- stimulate the immune responses against antigens/toxins
- elicit production of enzymes and metabolic substances that degrade toxins
- block attachment sites in the colon for the pathogenic bacteria

Probiotic agents, such as *Lactobacillus GG and Saccharomyces boulardii,* have been studied for the treatment of *Clostridium difficile.* The use of probiotics for primary and recurrent

CDAD treatment looks promising (13). In a study performed almost 20 years ago, it had been found that patients treated with a combination of *Bifidobacterium longum* yogurt and erythromycin had decreased stool clostridia spore counts versus patients who were treated with placebo and erythromycin (14). Standard antibiotic treatment of CDAD plus *L. rhamnosus GG* probiotic for 3 week reduced *C. difficile* infection with improved patient well-being and earlier disappearance of abdominal cramps and diarrhea (15, 16, 17). Good results have been received also in a single randomized, controlled trial using again *L. rhamnosus GG* with either metronizadol or vancomycin (14). *Lactobacillus plantarum* 299v has been tested in a small study of 20 patients with recurrent CDAD along with metronidazole for 38 days. Although the recurrence frequency has been lower in the group with the probiotic (36.4%) compared to the placebo group (66%), the researchers consider that the difference is not significant (18). There is a vast amount of published literature discussing the role of probiotics in antibiotic-associated diarrhea (AAD) and CDAD (19). Strong evidence that probitics significantly reduced the risk of developing AAD and CDAD was found in meta-analyses of 25 randomized controlled trials. The most studied *Saccharomyces boulardii* probiotic (20) has shown good results in treatment of CDAD, but this yeast-microorganism did not have impact on the recurrence rates of patients treated with low dose (less than 2 g vancomycin/day) antibiotic.

In conclusion, the use of probiotics to prevent and control CDAD is promising. The results from different trials are positive, in spite of the fact that in some cases they are inconsistent.

Based on the literature and our experience there are several factors that must be taken into account in the effort to achieve better results in controlling CDAD with probiotics:

A. Knowing the stage and the severity of CDAD as well as the pathogenic properties of the *C. difficile* strain causing the disease is very important from a clinical point of view. We should also have a better understanding concerning the pathogenic properties of the isolated strain and especially the type and amount of toxins released.

B. The status of the patient's immune system and ability to deal with infections should also be determined. In this respect the function of the GI tract and the liver is of utmost importance.
C. The type of the probiotic mixture is also very important because not all strains, even within the same type, have the same beneficial properties. The selection of the strains of Lactobacillus and Bifidobacteria and their inclusion in the probiotic formula are of paramount importance.

Potential Beneficial Effects of Probiotics on CDAD

- Competitive blockage of the attachment/receptor sites for *C. difficile* and its toxins in the intestines; thereby preventing adhesion and invasion of the pathogenic bacteria (21),. This is important for prevention and prophylaxis of CDAD and for the reestablishment of the beneficial/natural microflora that will restore the normal function of the GI tract after antibiotic treatment.
- Immune response enhancement by Lactobacillus and Bifidobacteria (22). Local effect of immunoglobulin A (IgA) antibodies against the *C. difficile* and the released toxins is important for the termination of the disease. Animal trials indicate that secretory IgA as well as a modification of the cytokine profile that enhances anti-inflammatory cytokines can be triggered by *Lactobacillus* ingestion (23, 24). Having in mind that the immune system of patients with CDAD needs a natural support, probiotic therapy is most likely the best modality for reconstitution of the enteric microbiota. In spite that most probiotics are sensitive to antibiotics, their use along with antibiotics may enhance the host's immune system significantly by providing inactivated antigens.
- Signal(s) from Lactobacilli to the host regulates the secretory and motility defenses designed to remove noxious substances (25). The glycosylation of the intestinal mucins (MUC2 and MUC3) is increased in response of lactobacillus signaling. This protects the intestinal cells against pathogenic bacteria adhesion (26). It is also postulated that lactobacilli regulate the release of nitric oxide from the enterocytes which affects the motility of the intestines (27).
- Lactobacilli and Bifidobacteria produce metabolic substances that affect positively the GI tract and other physiological systems of the human organism. The

probiotics produce lactic acid and short chain fatty acids which decrease the acidity of the intestinal content, making the intraluminal environment unfavorable for the pathogenic bacteria. Production of antibiotic like substances such as bacteriocins is another important metabolite. Production of amino acids, folates, vinamins and immune stimulating metabolic substances, all together contribute to the elimination of the intestinal infections.

Supernatant Synbiotic Formula

Composition:

Lactobacillus bulgaricus (multi strains), *Streptococcus thermophilus* (multi strains), *L. helveticus* 104, *L. acidophilus* 108, *L. casei* 115, *Bifidobacterium longum* 112, *B. infantis* 117, Supernatant (inactive cells of *L. bulgaricus* and *S. thermophilus* with metabolic substances released during fermentation of the strains), and Inulin.

Product characteristics:

Description	Powder
Preservatives	None

Microbiological Profile:

Lactic acid Bacteria Plate Count	CFU/g 45 billion
E. coli, Salmonella and Coag. Pos. Staph	Negative

Ingredient statement:

All component of the Supernatant Synbiotic Formula are proprietary of BioImmersion Inc. Bellevue, USA.

Package and Storage:

Capsules of 500mg each in a bottle stored at room temperature or refrigerated.

Shelf life:

Two years

Suggested Usage:

Use 1-2 capsules two times daily (morning and evening) with meals.

Advantages and expected benefits of the Supernatant Synbiotic Formula during control of CDAD

Having in mind the pathogenensis and nature of CDAD, the first step to control this infectious disease is to eliminate the source of infection in order to avoid introduction of carriers of *C. difficile.* In this regard, the use of low doses of the Supernatant Synbiotic is one of the prophylactic measures that should be recommended in the effort to prevent spread and recurrence of *C. difficile*.

For patients with diagnosed active CDAD, we propose to use the Supernatant Synbiotic Formula as an adjunctive therapy along with antibiotics and as an alternative therapy, alone which should be continued after completion of the antibiotic therapy for 4-6 weeks. The probiotic must be taken regularly during meals in the morning and in the evening. The dose of the probiotic should be adjusted depending on the severity of the CDAD.

The BioImmersion blend of *Lactobacillus* sp. and *Bifidobacterium* sp. is unique for several reasons:

- The strains *L. acidophilus, L. casei, L. helveticus, B. infantis and B. longum* colonize the GI tract well. They eliminate competitively colonization in the GI tract by pathogenic bacteria including *C. difficile.*
- By providing a prebiotic, such as inulin, to *Bifidobacteria*, the short-chain fatty acid formation increases and the intestinal pH decreases which makes the intestinal environment unfavorable for *C. difficile*. Short chain fatty acids are known to be the main nutritive resource for the enterocytes. Increased production of short chain fatty acid or enteric nutrients may improve the health of the lower GI tract.
- The bacteria in the Supernatant Synbiotic Formula releases antibacterial substances such as bacteriocines, which suppress the growth of pathogenic bacteria.

- The strains also produce metabolic substances such as vitamins, folic acid, folates, amino acids and other.
- The strains included in the formula have immunogenicity that helps regulate the immune responses during CDAD.
- The strains included in the formula help with absorption of liquids (decreasing symptoms of diarrhea) and minerals including sodium, which is responsible for watery stools.
- The Supernatant effect is based on immunomodulation caused by inactive cells from *L. bulgaricus* and *S. thermophilus*, along with metabolic substances formed during their fermentation. The main metabolic substances are: lactic acid, folates, vitamins, amino acids, bacteriocines, angiotenzin converting enzyme (ACE) inhibition peptides.

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