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Role of *Lactobacillus* Spp. in Treatment of Diarrheic Cases Caused by *Clostridium Difficile*

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Abstract

Background: Antibiotic treatment can disturb the resistance of the gastrointestinal flora to colonization. This may result in complications, the most serious of which is *Clostridium difficile* associated diarrhea (CDAD). The study was to determine the effectiveness of probiotics for the prevention of CDAD. *Clostridium difficile* is the most important cause of nosocomial diarrhea in adults. Illness may range from mild watery diarrhea to life-threatening colitis. Diagnosis is based primarily on the detection of *C. difficile* toxin A or toxin B. These toxins primarily disrupt the cytoskeletal structure and the tight junctions of target cells causing cell rounding and ultimately cell death. The toxins trigger a complex cascade of host cellular responses to cause diarrhea, inflammation and tissue necrosis- the major symptoms of CDI. Our findings indicate that probiotics may prevent CDAD. Most probiotics contain a singular strain. The combination with lactobacillus sp was the most effective at preventing CDAD. In addition, 6 out of 8 trials had an in relation to preventing CDAD containing *Lactobacillus* spp. Our findings indicate that probiotics may prevent CDAD. Most probiotics contain a singular strain. The combination with *Lactobacillus* spp. was the most effective at preventing CDAD. Conclusion: In addition, 6 out of 8 trials had an in relation to preventing CDAD containing lactobacillus sp. Four studies said that there were some factors that meant that the probiotic could not reduce or prevent the CDAD.

Keywords: CDAD, Toxin A, Toxin B, Gastrointestinal flora, Iraq

Introduction

Antibiotics are among the most prescribed medications worldwide. Antibiotic treatment may disturb the colonization resistance of gastrointestinal flora, resulting in a range of symptoms, most notably, and diarrhea. Antibiotic use results in a depletion of the normal microflora (MO), which allows *Clostridium difficile* (*C. difficile*) to more easily colonize and infect patients. The spectrum of *C. difficile*-related disease varies from asymptomatic intestinal colonization, diarrhea, colitis, and pseudo-membranous colitis to toxic megacolon and death [1]. Antibiotic exposure is considered the most significant risk factor for CDI, and several drugs have been implicated in high CDAD rates including cephalosporins, fluoroquinolones, penicillins, and clindamycin [2]. Other CDAD risk factors include the use of proton-pump inhibitors, H2 antagonists, methotrexate, and the presence of existing gastrointestinal pathologies, such as inflammatory bowel disease. Most approaches for the prevention of CDI to date have focused

on limiting the spread of CDI. The most common methods are early detection and isolation, contact precautions, and appropriate hand hygiene. A number of studies have focused on the role of environmental cleaning to eradicate CDI in the health care environment, including the use of environment disinfectants as well as chlorhexidine patient baths, but have shown limited success. More recently, probiotics have been proposed for the prevention and treatment of a variety of gastrointestinal conditions, including diarrhea. Normal intestinal flora is an important barrier against pathogenic bacteria, and disruption of this normal flora with antibiotic use can lead to diarrhea. Probiotics are live microbial food supplements and have been hypothesized to counteract disturbances in intestinal flora and reduce colonization by pathogenic bacteria [3]. Various species of probiotics have been studied, with the most common being within the Lactobacillus and *Bifidobacterium* genus. More recently, *Saccharomyces boulardii* has also been considered a probiotic. Probiotics are live organisms thought to improve the microbial balance of the host, counteracting potential disturbances in intestinal flora associated with antibiotic use, and reducing the risk of colonization by pathogenic bacteria. Probiotics are becoming increasingly available as capsules and dairy-based food supplements sold in health food stores and supermarkets with use among consumers increasing four-fold from 2007 to 20124 [4].

Clostridium difficile

Clostridium difficile is an anaerobic, gram-positive, spore-forming bacillus that first was isolated in 1935 from the fecal flora of healthy neonates [5]. In 1978, the association between cytotoxins released by this organism and antibiotic-induced pseudo-membranous colitis first was reported [6]. Since that time, the incidence of *C. difficile* infection has increased dramatically, and the organism now is recognized as the primary cause of nosocomial infectious diarrhea in developed countries [7]. Incidence rates of nosocomial infection range from 0.1 to 30 per 1000 patients in nonepidemic settings [8]. In community populations, the reported prevalence of *C. difficile*– associated diarrhea ranges from 8 to 12 per 100,000 person-years [9]. Knowledge of the epidemiology, pathogenesis, and treatment of disease caused by *C. difficile* was increased dramatically in the 1980s and 1990s. This increased knowledge has not led, as yet, to any substantial decline in the frequency of hospital-acquired *C. difficile* diarrhea and colitis.

Pathogenesis

Pathogenic strains of *Clostridium difficile* produce two potent exotoxins, enterotoxin (ToxA) and the cytotoxin (ToxB), (Figure 1).

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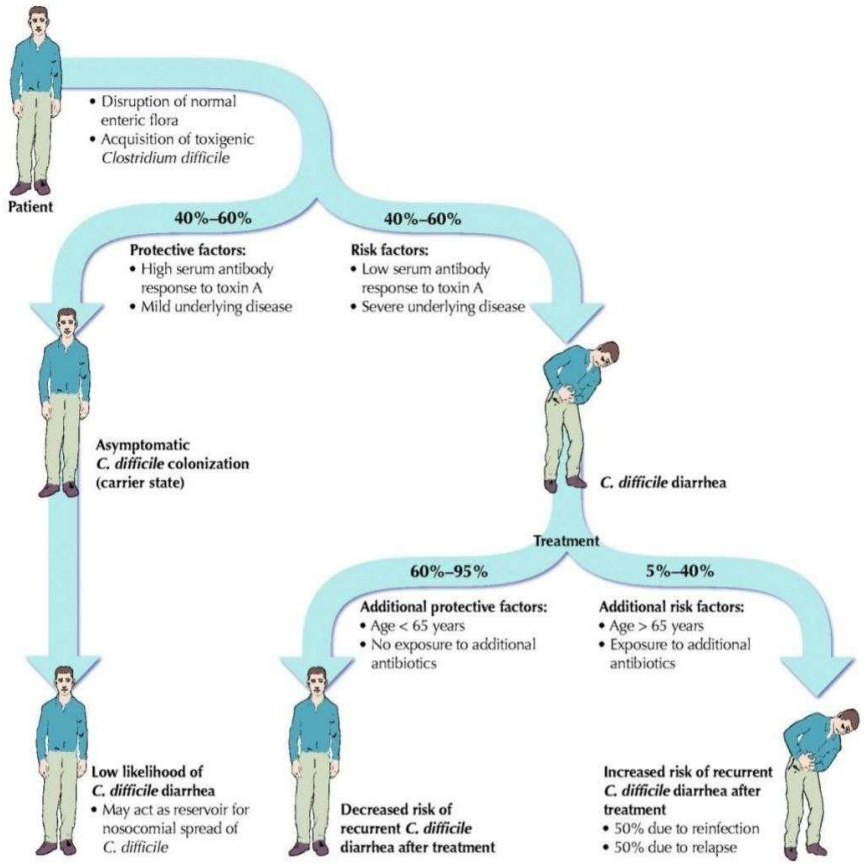

Figure (1): Pathogenesis of *Clostridium difficile*

Clostridium difficile is a Gram-positive, anaerobic, spore-forming, toxin producing bacillus [10]. *Clostridium difficile* is the most common cause of nosocomial infections in the United States surpassing methicillin resistant *Staphylococcus aureus* [11]. *Clostridium difficile* is acquired through ingestion of spores usually transmitted from other patients through the hands of healthcare personnel or the environment [12]. The spores resist the acidity of the stomach and germinate into the vegetative form in the small intestine. Disruption of normal gut flora typically by exposure to antimicrobials allows *C. difficile* to proliferate causing a broad spectrum of clinical manifestations that can range from asymptomatic carriage to diarrhea of varying severity to fulminate colitis and even death [13].

Clostridium difficile Toxins

Tox A causes hemorrhagic fluid secretion in the intestinal loop, mucosal inflammation and necrosis of the intestinal tissue (3-5). In contrast to ToxA, ToxB exhibits no overt enterotoxicity. However, both toxins are lethal when injected parenterally into animals [10]. Infection with *C. difficile* is responsible for *C. difficile* diarrhea. *Clostridia* are anaerobic motile bacteria, ubiquitous in nature and especially prevalent in soil. Under the microscope, they appear as long, irregular (often drumstick or spindle-shaped) cells with a bulge at their terminal ends [13].

Most *C. difficile* strains produce two major toxins, i.e., TcdA and TcdB, generated by the genes TcdA and TcdB within the organism's pathogenicity loci (PaLoc), while certain *C. difficile* strains may produce a binary toxin called *C. difficile* transferase (CDT) closely related to the

Clostridium perfringens binary toxin [11]. Additionally, the lethal and hemorrhagic toxins from *C. sordelli* toxin from *C. novyi* and the large cytotoxin from *C. perfrigens* belong to this family [14, 15]. However, the incidence of *C. difficile* infections related to strains only producing CDT is low and the symptoms are moderate. In addition, these strains yield no severe lesions of enteritis in experimental animal models [11, 14].

Management

Management of mild, moderate, and severe CDI

If a patient has strong a pre-test suspicion for CDI, empiric therapy for CDI should be considered regardless of the laboratory testing result, as the negative predictive values for CDI are insufficiently high to exclude disease in these patients (Strong recommendation, moderate-quality evidence). Any inciting antimicrobial agent(s) should be discontinued, if possible (Strong recommendation, high-quality evidence). Patients with mild-to-moderate CDI should be treated with metronidazole 500 mg orally three times per day for 10 days (Strong recommendation, high- quality evidence). Patients with severe CDI should be treated with vancomycin 125 mg four times daily for 10 days (Conditional recommendation, moderate-quality evidence). Failure to respond to metronidazole therapy within 5–7 days should prompt consideration of a change in therapy to vancomycin at standard dosing [16].

Management of severe and complicated CDI

Supportive care should be delivered to all patients and includes intravenous fluid resuscitation,

electrolyte replacement and pharmacological venous thromboembolism prophylaxis. Furthermore in the absence of ileus or significant abdominal distention, oral or enteral feeding should be continued (Conditional recommendation, low-quality evidence). CT scanning of the abdomen and pelvis is recommended in patients with complicated CDI (Conditional recommendation, low- quality evidence). Vancomycin delivered orally (500 mg four times per day) and per rectum (500 mg in a volume of 500 ml four times a day) plus intravenous metronidazole (500 mg three times a day) is the treatment of choice for patients with complicated CDI with ileus or toxic colon and significant abdominal distention. (Strong recommendation, low-quality evidence)

Management of recurrent CDI (RCDI)

No effective immunotherapy is currently available. Intravenous immune globulin (IVIG) does not have a role as sole therapy in treatment of RCDI. However, it may be helpful in patients with hypogammaglobulinemia (Strong recommendation, low-quality evidence). The first recurrence of CDI can be treated with the same regimen that was used for the initial episode. If severe, however vancomycin should be used. - The second recurrence should be treated with a pulsed vancomycin regimen (Conditional recommendation, low-quality evidence). If there is a third recurrence after a pulsed vancomycin regimen, fecal microbiotatransplant (FMT) should be considered (Conditional recommendation, moderate quality evidence).

Management of patients with CDI and co-morbid conditions

In patients who have IBD with severe colitis, simultaneous initiation of empiric therapy directed against CDI and treatment of an IBD fl are may be required while awaiting results of

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C. difficile testing (Conditional recommendation, low-quality evidence). All patients with IBD hospitalized with a disease are should undergo testing for CDI (Strong recommendation, high-quality evidence).

Risk factor

Pharmacological risk factors include any use of antibiotics (broad and specific), any use of proton pump inhibitors, any use of histamine 2 receptor antagonists, anti–ulcer medications (not specific), non-steroidal anti–inflammatory drug, aspirin, corticosteroids and using of opiate during the last episode of CDI.

Host-related risk factors as age, chronic kidney disease, diabetes mellitus, lymphoma or leukemia, solid cancer or malignancy, severity of co-morbidity, inflammatory bowel disease, congestive heart disease, chronic obstructive pulmonary disease, peptic ulcer, previous diagnosis of CDI. Clinical interventions or characteristics: duration of hospitalization, nasogastric tube feeding, stay in intensive treatment unit, non–surgical GI procedure, vomiting, history of surgery, previous gastrointestinal procedure, and serum albumin [17].

Treatment

Treatment of a first episode of recurrent infection with a repeat course of either metronidazole or vancomycin for 10 to 14 days is successful in approximately 50% of patients. Second and subsequent recurrences can be difficult to cure, primarily because of the persistence of spores in the bowel or environment and the inability of the patient to mount an effective immune response to *C. difficile* toxins rather than to antibiotic resistance. Second recurrences can be treated with fidaxomicin or by a vancomycin regimen involving tapered and pulsed dosing [12, 11]. Metronidazole and oral vancomycin have been the mainstays of treatment for *C. difficile* infection since the 1970s. For the treatment of severe disease, vancomycin is better than metronidazole but for mild-to-moderate infection the two antibiotics have been considered to be equivalent. However a marked rise in clinical failure associated with metronidazole especially in patients with the BI/NAP1/027 strain, has been seen in the past decade. Previous studies were underpowered to evaluate differences between metronidazole and vancomycin in cases of no severe infection, but recent data suggest an overall superiority of vancomycin.

Probiotic

Probiotics are live M.O that, when taken in sufficient quantities, help the host health by restoring the microbial balance in the body [18]. Probiotics that as live MO that, when administered to a host in sufficient amounts, confer health benefits [19]. The term "probiotics" is derived from the Greek word meaning "for life". Elie Metchnikoff developed the concept of probiotics at the Pasteur Institute in Paris at the end of the 19th century. Scientists have been attempting to clarify and define the probiotic effect ever since [20]. Furthermore, Abriouel et al. (2012) examined that LAB creates part to the autochthonous microbiota of a variety of foods, such as yogurt, sour cream, sausages, olives, and others [21]. They are defined as a set of lactic acid producing, low G+C percentage MO. Gram positive cocci or rods, non-spore forming bacteria, and catalase-negative bacteria share many biochemical, physiological, and genetic properties [22]. The probiotics are growing rapidly due to mounting scientific evidence of their positive benefits on human health [23].

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Characteristics of a probiotics

An effective probiotic isolate should have features, such as persistence and colonization ability under a variety of environmental circumstances. The isolates should be able to tolerate bile salts, low gastric juice pH, and adhering to epithelial cells [24]. They must also provide demonstrable health benefits, such as anticancer, antimicrobial, toxin-reducing, and immune-boosting properties. As a result of microbial adhering to surfaces and surviving in the gastrointestinal tract GIT should be tested in vitro before being used as probiotics [25]. They must also be able to stimulate local metabolic action (26). Furthermore, probiotics should be consistent, safe, active, and capable of remaining usable for extended periods of time under storage conditions, and it must be capable of restoring and replacing intestinal micro-flora [27].

Mechanism action of probiotics

Probiotics are known to act strain-dependently and inhibit pathogenic bacteria via various mechanisms, as reported in various studies [28]. Probiotics have various mechanisms of action; currently, three major steps of probiotic action have been demonstrated:

A) Nutrient and ecological element competition, at this point, the anaerobic flora in the digestive tract limits the concentration of potentially pathogenic flora, probiotics can directly affect other M.O by inhibiting pathogen adhesion, this type of major defense mechanism is used to remind internal health conditions, *Lactobacilli* and *Bifidobacterium* spp. have been shown to inhibit a wide range of pathogens by colonizing pathogenic bacteria and forcing antagonistic activity against GIT pathogens.

B) Synthesis of antimicrobial substances, such as organic acids (lactic and cetric acid), toxins, and bacteriocins which lower the pH of the GIT, this substance is responsible for inhibiting pathogen growth, which leads to pathogen death by creating antagonistic conditions, and some action may occur in the inactivation of toxin.

C) Immune response stimulation or modulation by T-cell activation to cytokine production throughout immune deletion by persuading phagocytosis and IgA sectarian, modifying response Th1 enhancing and Th2 suppressing responses, this mechanism of action is crucial in the inhibition and treatment of infectious illnesses [29].

Inhibitory compounds produce by probiotic

Prabhurajeshwar and Chandrakanth (2019) clarified that Lactobacillus spp. that produce antibacterial substances, such as lactic and additional organic acids (lactic and citric acids), hydrogen peroxide (H_2O_2) bacteriocins, and others that are low-molecular weight proteins or peptides have biologically active that prevent the growth of a variety of pathogenic bacteria. Probiotic strains must not compete with the normal MO; rather, they must interconnect in symbiotic relationships [30]. Furthermore, *L. acidophilus* produces antimicrobial compounds such as acidophilin, bacterial peptides, and lactic acid. The probiotic is more safe and beneficial than antibiotics; the probiotic inhibited the growth of unwanted M.O without the danger of developing resistance pathogenic organisms or interfering with human normal flora; antibiotics are frequently used, which leads to the growth of multidrug resistant MO [31]. The usage of live MO has a positive effect on human intestinal micro-flora, longevity, and enhance human health; the usage of lactobacilli over a long period of time saves human exposure, and with

very few minor public health problems, most lactobacilli strains have been categorized as "generally recognized as safe [32].

Advantages of probiotic

Patients prefer medicines with few or no side effects for the treatment of their illness, and probiotics provide such an alternative choice. Because they are living, non-harmful MO, generally recognized as safe, indicating that they are extremely safe [33]. Probiotics MO are used to treat and prevent conditions, such as lactose intolerance, diarrheal disorders, allergies, lowing cholesterol level, decreasing the risk of carcinogenic effect, and immune system modulation. Furthermore, Shiel et al. (2004) reported that probiotics have been shown to have beneficial effects not only in the stomach, but also in other parts of the body. Probiotics, for example, have been shown to have anti-inflammatory properties when administered parenterally. Lactose absorption problems (also known as lactose intolerance) are caused by a lack of the enzyme -D-galactosidase, which prevents lactose from being hydrolyzed (lactase). Lactose that hasn't been digested finds its way to the colon, where lactose fermenters break it down (Adams and Moss, 2000). Lactose fermentation in the colon results in high levels of glucose in the blood and hydrogen gas in the breath.

Probiotics must meet a number of basic requirements in order to expand into marketable probiotic products. The most important requirements are that bacterial probiotics survive in sufficient numbers in the product and that their genetic and physical stability is guaranteed throughout product storage [34]. All of their properties are critical for expressing their health benefits after consumption and must be retained during product production and storage [35]. Furthermore, Parvez et al. (2006) proposed that the activity of microbial gut bacteria, particularly beneficial cultures, could improve the bioavailability, digestibility, and amount of certain nutrients. Probiotic consumption has been associated with greater levels of niacin, riboflavin, folic acid, vitamin B6, vitamin B12, and thiamine production.

Probiotics play an important role in increasing iron, calcium, manganese, phosphorus, and copper bioavailability in yoghurt, as well as fat and protein digestibility [36]. When enzymes hydrolyze fat and protein, short chain fatty acids and free amino acids are produced. Organic acids produced by LAB during fermentation, such as lactate and acetate, lower the pH of intestinal substances, creating an unfavorable environment for pathogenic bacteria [37]. Other advantages of probiotics include the reduction or elimination of disorders, such as colon irritation, constipation, and traveler diarrhea, as well as the inhibition of harmful bacteria. When the colon contains an adequate amount of probiotics, the production of β-galactosidase improves lactose absorption [38].

Disadvantages of probiotic

Manipulation of the gut microbiota is difficult and might result in bacterial-host interactions [39]. However, probiotics are generally found to be safe, some people are concerned about their safety in specific situations. Immunocompromised patients, those with short bowel syndrome, central venous catheters, and heart valve disease, as well as premature infants, may be more vulnerable to various effects. As a result of bacteremia, there is a danger of live bacteria passing from the GIT to the internal organs (bacterial translocation), which can have negative health

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repercussions in very unwell persons with inflammatory bowel disease [40]. Probiotics may cause bacteremia or fungemia (bacteria or fungi in the blood), which can lead to sepsis, a potentially fatal condition, in children with a weakened immune system or who are already critically sick [41].

Probiotics affecting C. Defficile associated diarrhea

A combination probiotic treatment was associated with significant *C. defficile* associated diarrhea on these studies. Eight trials reported a preventive effect against CDAD with a mixture of Lactobacillus casei and Lactobacillus paracasei CNCM I-1518 and a mixture of 4 strains containing *Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium* lactis Bi-07 and B. lactis Bl-04 [42-45]. There was also a mixture of Lactobacillus helveticus R0052, *Lactobacillus rhamnosus* R0011 and *Saccharomyces boulardii* CNCM I-745 (SB) [46- 48]. VSL#3 contains a mixture of *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii* spp., *Bulgaricus, Streptococcus thermophiles, Saccharomyces boulardii* and *Lactobacillus casei* [49-52]. Six studies reported that *Lactobacillus* spp. was the most effective probiotic at preventing *C. defficile* associated diarrhea. Across the 4 trials, it was reported there was no important impact on Clostridium defficile associated diarrhea when using a mixture of 2 strains, namely *Lactobacillus acidophilus* (CUL600 and CUL21) and two strains of *Bifidobacterium* (*Bifidobacterium bifidum* CUL20 and *Bifidobacterium lactis* CUL34). There was also a mixture examined consisting of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12, *Saccharomyces boulardii* and ACTIMEL containing *Lactobacillus casei, Lactobacillus bulgaricus* and Streptococcus thermophiles [53-56].

Table 2.1 Multi strain or single strain Probiotic Genus

Author	*Lactobacillus* spp.	*Bifidobacterium* spp.	*Streptococcus* spp.	*Saccharomyces* spp.	Multi strain	Single strain
Alberda et al., 2018				√		√
Allen et al., 2013	√	√			√	
Barker et al., 2017	√	√			√	
Chatterjee et al., 2013	√	√			√	
Ehrhardt et al., 2016				√		√
Evans et al., 2016	√				√	

Kabbani et al., 2017	√					√
Mallina et al., 2018	√		√		√	
Ouwehan d et al., 2014	√	√			√	
Selinger et al., 2013	√	√	√		√	
Shan et al., 2013				√		
Wong et al., 2014	√					√

Results

The age of the participants in the studies ranged between 18 and 70 years. The average age was adult. There were 7 studies that reported on a combination of genera and 5 studies didn't report what they used. The probiotics which prevented CDAD were from 4 major genera. There was *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp. and *Saccharomyces* spp. The probiotics used included from within the Lactobacillus sp genus included *Lactobacillus casei*, *Lactobacillus acidophilus* (CUL60, CUL21, LA-5, NFCM), *Lactobacillus paracasei* (Lpc-37), *Lactobacillus helveticus* R005, *Lactobacillus rhamnosus* R0011, Lactobacillus bulgarius and *Lactobacillus casei Shirota*. The probiotics used that were *Bifidobacterium* sp included *Bifidobacterium bifidium* (CUL20, W23), *Bifidobacterium lactis* (CUL34, Bi-07, B1-04, BB-12), *Bifidobacterium breve*, *Bifidobacterium longum* and *Bifidobacterium infantis*. The probiotics used that were *Streptococcus* sp included *Streptococcus thermophiles* and Streptococcus boulardii. The probiotics used that were *Saccharomyces* spp. included *Saccharomyces boulardii* and *Saccharomyces* CNCM I-745. Duration of probiotics giving in this studies was varies, it about from 7 days to several weeks. The doses of the probiotics in the studies varied, and they ranged from a minimum of 1.0×10^7 cfu to a maximum dose of 6×10^{10} cfu. Other preparations include 93 ml, 2 techsules, 250 mg, 100 gm (97 mL) and 2 sachets. The duration of the probiotics and antibiotics varied. Diarrhea was defined as consisting of 2 main variations, which were ≥ 3 loose stools in 24 h and ≥ 2 loose or watery stools per day.

Discussion

The result of this review found that the age group most affected by CDAD was adults (the youngest being 6 months through to old age in the study overall), although this was not statistically significant. One study stated that being of an age >18 years old may increase the risk by about 2% concerning being infected by *C. defficile* in the health care setting. However, the level of infection was neither studied nor evaluated deeply. Old age individuals are more susceptible to *C. defficile* infection because it is related to their humoral immune response [45].

In addition, CDAD infection often happens at an old age where, in the health care setting, they have consumed broad spectrum antibiotic [42]. Based on the review, the duration of consumed probiotics in the studies varied and it was between a minimum of 7 days to a maximum of several weeks. The other studies said that a short treatment duration (≥8 weeks) in reference to bowel inflammation. This is related to the quality of the patient's life because longer term or even the continuous supplementation of probiotics may be required to detect significant alterations in the symptoms [46]. We found 4 journals that said that probiotics cannot reduce CDAD. This was found because ineffective probiotic results were related to the limitations of the trial.

Another thing to consider is that the number of study participants was 80.5% for those not eligible and the patients who were eligible may have been relatively healthy. One must consider a different design for the trial to get better results [43]. On the other hand, our review showed that the probiotics that can reduce CDAD were from 4 different genera. These were Lactobacillus, *Bifidobacterium*, *Streptococcus* and *Saccharomyces*. It also was explained by Johnston and colleagues. Their systematic review consisted of 20 RCTs with 3818 patients to determine if probiotics are effective at preventing CDAD. They found evidence that shows that probiotics reduce the chance of CDAD to a large extent, with only a small percentage of adverse reactions. The probiotics used were *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, and Streptococcus [47]. It was explained that probiotics are the most effective if given closer to the first antibiotic dose, with a decrement in efficacy for every day of delay in starting probiotics [48]. Probiotics *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, and *Streptococcus* may have effects that can be attributed to its actions on intestinal immunity. They may improve the number of IgA and other immunoglobulin secreting cells in the intestinal mucosa and it can also stimulate the local release of interferons. It could also function through the advancing of the barrier function, immune- modulation, and competitive adherence to the intestinal mucosa by avoiding or ameliorating various infective or inflammatory diseases [49].

On the other hand, this review has shown that Lactobacillus is a great species determinant for the prevention of CDAD. For example, *Lactobacillus casei* becomes practical when it is flavored. It was shown by Alberda teams [50] said that 32 participants in trial. AAD was documented in 12.5% of the probiotic *Lactobacillus casei* drink group and 31.3% in the control group. Most of the studies stated that probiotics were more effective against bacterial diarrhea. For instance, when the efficacy of Lactobacillus GG was analyzed in a meta-analysis, as for separate etiologies, it was evident that this probiotic was most effective for rotavirus diarrhea [51]. One trial said that Lactobacillus sp strains have been shown to survive passage through the gastrointestinal tract when healthy volunteers were given *Eubacteriaceae*, causing diarrhea. In in-vitro studies, these strains have shown the ability to adhere to human epithelial cells, to maintain the gut barrier and to stimulate an anti-inflammatory response, in addition to blocking pathogen adhesion. It is feasible that these mechanisms have a role in reducing the duration of diarrhea events [52]. *Lactobacillus* can also reduce CDAD according to Ouwehand et al. as seen in a trial. Their result showed there to be a significant dose response effect in CDAD with an incidence of 12.5, 19.6, and 24.6 with the high dose, low dose and placebo ($p=0.02$). They said that abdominal pain was reduced only in the high-dose group, focusing on the diarrhea cases. Only the low dose group showed a trend for reduced abdominal pain. This reduction in

pain is interesting, as *L. acidophilus* NCFM, one probiotic out of the components in the tested preparation shown earlier, was shown been able to increase the pain threshold in rats by inducing the expression of the μ opioid and canabinoid 2 receptor numbers. Both the average of the liquid stools and the average duration of the diarrhea were significantly reduced by both the high and low doses compared to the placebo [53]. The other study said that the average of the probiotics containing Lactobacillus sp. had a preventive effect on CDAD, with a pooled relative risk reduction of 75 [54]. The limitation in this study was that statistical evidence was not provided to support the recommendations for the routine using of microbial preparations for CDAD prevention. The most effective probiotics preparations or probiotic forms to prevent CDAD still need to be investigated [55].

Conclusion

This systematic review was used to determine the effectiveness of probiotics at preventing CDAD. Our findings indicate that probiotics may prevent CDAD. Most probiotics contain a singular strain, but it was the one that was in combination with Lactobacillus sp that was the most effective at preventing CDAD. In total, 6 out of 8 trials showed as having an effective effect when it comes to preventing CDAD containing Lactobacillus sp. Four studies said that there were some factors that meant that the probiotic cannot reduce or prevent CDAD: this includes giving probiotics that are not according to the Doctor's recommended dosage and not only consuming the probiotic but also high dosages of antibiotics

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