Beneficial Microbes, June 2013; 4(2): 211-216

Wageningen Academic Publishers

Efficacy of *Bacillus clausii* strain UBBC-07 in the treatment of patients suffering from acute diarrhoea

M. Ratna Sudha[1], S. Bhonagiri[2] and M. Asin Kumar[3]

[1]Centre for Research & Development, Unique Biotech Limited, SP Biotech Park, Phase-II, Plot-2, Shameerpet, Hyderabad 500078, AP, India; [2]Medipoint Hospital Pvt. Ltd. Pune, Maharastra 411007, India; [3]Department of Medicine and Nephrology, Government LTMG Hospital, Sion, Mumbai, Maharastra, India; sudha.ratna@gmail.com

Received: 11 June 2012 / Accepted: 15 November 2012

Abstract

This study was conducted to evaluate the anti-diarrhoeal activity of *Bacillus clausii* strain UBBC 07 in patients suffering from acute diarrhoea. A total of 27 patients (average age of 35.44±8.08 years) with acute diarrhoea were included in a prospective, Phase II clinical study after informed consent and ethical committee approval. The criteria included for all subjects were ≥3 loose stool motions within 24 hours and for more than 7 days. All patients were assigned to receive one capsule of *B. clausii* strain UBBC-07 (containing 2×10^9 cfu) two times a day for a period of 10 days. Efficacy assessment of duration of diarrhoea, frequency of defecation, abdominal pain and stool consistency were tested on days 1, 3, 6 and 10. Safety was evaluated by assessing the incidence and type of adverse effects such as increase in blood pressure and pulse rate, physical examination and clinical laboratory tests, i.e. complete blood count, serum glutamic pyruvic transaminase, serum creatinine, and stool examination and microscopy, on day 1 and day 10. The results of this study clearly showed that the mean duration of diarrhoea decreased from 34.81±4.69 to 9.26±3.05 (P<0.0001) min per day, the frequency of defecation also decreased from 6.96±1.05 to 1.78±0.50 (P<0.0001) times per day, abdominal pain decreased from 3.22±0.93 (severe) to 0.74±0.71 (absent) (P<0.0001), and stool consistency improved from 3.93±0.38 (watery) to 1.22±0.42 (soft) (P<0.0001). No significant change in safety parameters were observed during treatment. This study shows that the *B. clausii* strain UBBC-07 can potentially be effective in alleviating the symptoms of diarrhoea without causing any adverse effects.

Keywords: *Bacillus clausii*, anti-diarrhoea, probiotics

1. Introduction

According to the World Health Organization (WHO, 1990), diarrhoea is defined as the passage of three or more loose or liquid stools within a 24-hour period. Diarrhoea is 'acute' if it resolves within 2 weeks, 'persistent' if it lasts for 2-4 weeks and 'chronic' if it lasts for more than 4 weeks. Acute diarrhoea is commonly diagnosed in adults and is responsible for cause of considerable morbidity around the world. Mortality due to acute diarrhoea is uncommon and occurs only during epidemics of diarrhoea. In industrialised countries, the incidence of acute diarrhoea was observed to be an average of 0.5 to 2 episodes per year per person. The incidence is higher in developing and underdeveloped countries than in industrialised countries (Manatsathit *et al.*, 2002).

While viruses are the major causative agent in children, both bacterial and viral pathogens are implicated in adults (Casburn-Jones and Farthing, 2004). Other causes for acute diarrhoea include irritable bowel syndrome, intake of some types of drugs, and ileal bile salt malabsorption. The treatment of acute diarrhoea mainly involves the prevention of dehydration, shortening the length of the illness, and reducing the period that a person is infectious (DeVrese and Marteau, 2007). Treatment with oral rehydration solutions (ORS) have significantly reduced the incidence of mortality and morbidity caused by diarrhoea, however,

ORS neither shortens the duration of diarrhoea nor stool consistency. Other treatment options include antibiotics, gut motility suppressing agents (e.g. loperamide, codeine), and probiotics (Casburn-Jones and Farthing, 2004).

Normal gut microbiota plays an important role in the protection of the host against gastrointestinal tract diseases (Fuller, 1991; Salminen and Deighton, 1992). During acute diarrhoea, the normal gastrointestinal microbiota is found to undergo radical changes that facilitate the overgrowth of unwanted microorganisms, including pathogenic strains. Several authors have reported that the administration of probiotics can restore the gut microbiota and also control the severity of diarrhoea (Johnston *et al.*, 2006; Shornikova *et al.*, 1997). Most of the studies have suggested that *Lactobacillus* spp., *Bifidobacterium* spp. and *Saccharomyces boulardii* could be effective in the treatment of diarrhoea (Sazawal *et al.*, 2006). However, species of *Bacillus* were also used commercially as probiotics as they possess potential probiotics properties (Ratna Sudha and Bhonagiri, 2012). A recent study by Hoyles *et al.* (2012) highlighted that most of the aerobic actinobacteria associated with the human gastrointestinal tract are dominated by *Bacillus* spp. such as *Bacillus clausii* and *Bacillus licheniformis.* Strains of *B. clausii* have been found to modulate immunity and prevent recurrent respiratory infections (Ciprandi *et al.*, 2005; Marseglia *et al.*, 2007). One of the advantages of using *Bacillus* spores is their ability to maintain viability over longer period of time (Mazza, 1994). *Bacillus* spores are resistant to gastrointestinal tract conditions, have the ability to germinate in the small intestine and also modulate the immunity of the host (Casula and Cutting, 2002; Duc *et al.*, 2003a). Maathuis *et al.* (2010) and Hatanaka *et al.* (2012) reported that the germination of *Bacillus* spores in gastrointestinal conditions is dependent on the dietary components that trigger the germination. *B. clausii* has been used widely as a probiotic for the treatment of diarrhoea in children and also for antibiotic associated side-effects (Benoni *et al.*, 1984).

B. clausii strain UBBC-07 (MTCC 5472) was isolated from soil contaminated with human faeces and identified by 16S rRNA sequencing. The strain was further characterised *in vitro* for acid and bile tolerance, cell surface hydrophobicity and antagonistic activity, and the results of *in vitro* evaluation showed that this strain possessed potential probiotic properties. The aim of the present study was to evaluate the effect of consumption of capsules containing *B. clausii* strain UBBC-07 (2×10^9 cfu/capsule) in patients suffering with acute diarrhoea.

2. Methods and materials

The study was conducted at the Kasturba hospital and Life Veda clinic (Mumbai, India) and the Medipoint hospital (Pune, India). All subjects with acute diarrhoea that fulfilled the selection criteria were invited to participate in an open label, Phase II clinical trial at these clinics.

Participants and eligibility

The eligibility to participate was having ≥3 loose stool motions within 24 hours for <7 days. The subjects participated after signing an informed consent form. They were withdrawn from the study for any of the following reasons: (1) serious adverse events, where continuation in the study posed a serious risk to the patient; (2) the patient had consumed less than 80% of the total dose and patients that did not take the capsules for more than 2 days consecutively; and (3) the subject consumed any of the prohibited medication (see below). When a subject withdrew before completing the study, the reasons for withdrawal were documented on the case report form and in the source document. When a subject was lost to follow-up, every possible effort was made by the study centre personnel to contact the subject and determine the reason for discontinuation. All follow-up measures were documented.

Upon enrolment, each patient was assessed on the duration and severity of diarrhoea, clinical features, i.e. fever, vomiting and dehydration, and nutritional status was established (any previous treatment also recorded). Subjects were provided with a reporting form to record clinical data. They were instructed to record daily the number of faecal outputs and consistency, symptoms of vomiting and fever, and checked for any necessity for hospital admission. The patients did not undergo any special change in dietary habits, medication, lifestyle, or exercise schedule after enrolled in the study. During the course of the study, the patients were not given any medication other than the studied probiotic preparation for diarrhoea. However, rescue medications like anti-motility drugs (loperamide hydrochloride, imodium and atropine diphenoxalate) were allowed in cases of acute emergency at the discretion of the investigator. Patients who were required to take medicines for any other complaints during the course of the study were allowed to take medicines only after consultation with the investigator and a record was maintained in the case report form. During the study, the patients were not allowed to use any other probiotic supplements, antidepressants, antipsychotic or appetite suppressant medicines, or tryptophan (to aid sleep), weight altering medications including homeopathic, ayurvedic, herbal (especially St. John's wort), Tibetan or unani preparations, or systemic steroids (chronic use, more than 2 weeks).

Treatment

A preparation of spores of *B. clausii* strain UBBC-07 (2×10^9 cfu/capsule) was administered orally for ten days in the form of two capsules a day before lunch and dinner.

Efficacy assessment

The primary outcome measures were the total duration of diarrhoea (in minutes), the frequency of defecation (number of stools per day). Stool consistency was evaluated through a score system graded as 1 (normal), 2 (loose), 3 (semi-solid), and 4 (liquid). Abdominal pain was scored as well and evaluated on a scoring system graded as 0 (absent), 1 (mild), 2 (moderate), 3 (severe) and 4 (very severe).

Safety assessment

Secondary outcome measures were the use of rescue medication and adverse events. Laboratory tests like haematological (complete blood count, serum glutamate pyruvate transaminase), biochemical analysis (serum creatinine) and stool examination were carried out as described previously (Ratna Sudha *et al.*, 2012) at the baseline (day 1) and end of the treatment (day 10).

Statistical analysis

Data for each patient were retrieved after completion of all their visits. The data obtained during the study were subjected to statistical analysis like t-test and chi-square test for comparing the results between the baseline and at the end of treatment. The significance level was taken at 95% level (P<0.05).

3. Results

A total of 27 (15 males and 12 female) subjects (treated with *B. clausii* strain UBBC-07) were enrolled in the study. The mean age of the subjects in this study was 35.44±8.08 years. No clinically relevant medical history was reported for any study subjects.

Safety assessment

Physical examination

There were no significant changes from the baseline in mean values for pulse rate, blood pressure (BP), temperature, respiratory rate and weight. However, a significant increase in diastolic BP was observed (P=0.0005) (Table 1), which is found to be in the normal range (BP: 120/80).

Clinical laboratory evaluation

Haematology and serum chemistry examination were carried out at baseline and day 10. Laboratory data are given in Table 2. No clinical significant changes in haematology and serum chemistry were observed during the study.

Stool examination

Stool analysis was done on day 1 and at the end of the study (Table 3). Stool examination detected the presence of fat on day 1 in the majority of subjects (22/27). However, all the subjects showed an absence of stool fat on day 10. Most of the subjects (24/27) showed mild mucus on day 1, which was resolved completely by day 10. Occult blood in stools was found in most of the subjects (22/27) on day 1; this was resolved completely on day 10. The presence of red blood cells (RBC) (22/27) and white blood cells (WBC) (23/27) in stools was observed at the beginning of the treatment and was found to be absent after the completion of the treatment. Three subjects were reported to have cysts of *Histocolytica* (amoebic parasite) in their stools on day 1; these were completely eliminated by the end of treatment.

Efficacy assessment

Efficacy assessment using parameters like duration of diarrhoea, frequency of defecation, abdominal pain, consistency of stool and fever was performed on days 1, 3, 6 and 10. Efficacy assessment parameters are summarised in Figure 1. Mean values of duration of diarrhoea decreased significantly (P<0.0001) from 34.81±4.69 on day 1 to 9.26±3.05 min per day on day 10; frequency of defecation decreased from 6.96±1.05 on day 1 to 1.78±0.50 (P<0.0001) times per day on day 10; abdominal pain decreased from 3.22±0.93 (severe) on day 1 to 0.74±0.71 (absent) on day 10 (P<0.0001); stool consistency improved from 3.93±0.38 (watery) on day 1 to 1.22±0.42 (soft) on day 10 (P<0.0001).

Table 1. Physical examination of subjects at baseline and day 10 of treatment (mean value ± standard deviation).

Variable	Day 1	Day 10	*P*-value
Pulse rate (beats per min)	74.89±4.65	73.56±3.25	0.0709
Systolic blood pressure (mm Hg)	123.70±12.44	120.80±7.84	0.0764
Diastolic blood pressure (mm Hg)	75.93±5.75	79.48±3.26	0.0075
Temperature (°C)	37.31±1.14	36.97±0.12	0.0112
Respiratory rate (no. of breaths taken per min)	18.30±2.39	18.78±1.96	0.1624
Weight (kg)	58.22±5.24	58.15±5.29	0.3265

Table 2. Mean values of haematology and serum chemistry of subjects at baseline and day 10 of treatment.

Variable[1]	Day 1	Day 10	*P*-value
Haemoglobin[2] (g/dl)	12.67±1.01	12.26± 0.82	0.0004
Total RBC (10^6/µl)	4.32±0.60	4.15±0.57	0.0205
Total WBC[2] (cells/mm^3)	7,503.00±969.727	7,162.96±1,123.233	0.0005
Eosinophils[2] (%)	1.89±1.15	1.30±0.99	0.0006
Basophil (%)	0.44±0.50	0.52±0.50	0.5735
Neutrophil (%)	69.85±5.30	68.26±6.46	0.1244
Monocytes (%)	3.81±2.84	3.22±2.73	0.0881
Lymphocytes (%)	24.00±6.42	26.70±7.31	0.0088
MCH (pg/cell)	28.04±1.74	27.89±2.39	0.7177
PCV[2] (%)	45.67±2.43	47.07±1.97	0.0038
Platelets	2.21±0.41	2.09±0.27	0.0152
Serum creatinine[2] (mg/dl)	1.35±0.49	1.16±0.41	0.0009
SGPT (U/l)	23.70±20.99	20.89±9.64	0.2279

[1] RBC: red blood cells; WBC: white blood cells; MCH: mean corpuscular haemoglobin; PCV: pack cell volume; SGPT: serum glutamic pyruvic transaminase.
[2] Range of normal values for healthy individual for haemoglobin (males 14.0-17.5 g/dl; females: 12.3-15.3 g/dl); total WBC (males and females 4,500-10,000 cells/mm^3); eosinophils (male and females 1-3%); PCV (males 42-52% ; females: 35-47%); Serum creatinine (males 0.7 to 1.3 mg/dl; females 0.6 to 1.1 mg/dl).

Table 3. Examination of patients' stools during the study.

Variable[1]	Scale	No. of subjects (%)	
		Day 1	Day 10
Stool fat	absent	5 (19%)	27 (100%)
	present	22 (81%)	0 (0%)
Stool mucus	absent	3 (12%)	27 (100%)
	present	24 (88%)	0 (0%)
Occult blood in stool	absent	5 (19%)	27 (100%)
	present	22 (81%)	0 (0%)
RBC in stool	absent	22 (81%)	27 (100%)
	present	5 (19%)	0 (0%)
WBC in stool	absent	4 (15%)	27 (100%)
	present	23 (85%)	0 (0%)
Ova and parasites (cysts of *Histocolytica*)	absent	24 (88%)	27 (100%)
	present	3 (12%)	0 (0%)

[1] WBC: white blood cells; RBC: red blood cells.

4. Discussion

Diarrhoea occurs mainly due to microbial (bacterial or viral) infections, antibiotic usage, tube feeding of patients and immune-compromised subjects (DeVrese and Marteau, 2007; Isolauri, 2003). The incidence of acute diarrhoea due to microbial infections is still a major health problem worldwide and a frequent cause of death especially in hospitalised children. Antibiotic associated diarrhoea is also a common clinical problem, which occurs due to the disturbance or destruction of indigenous microbiota caused by antibiotic treatment as well as subsequent overgrowth of normal microbiota, e.g. *Clostridium difficile* (DeVrese and Marteau, 2007).

The consumption of *Bacillus* spores was found to modulate the immunity. These spores may also interact with the host cells or microflora to enhance the potential probiotic effect (Duc *et al.*, 2003a,b). Another study demonstrated that the consumption of *B. clausii* reduced the common side effects

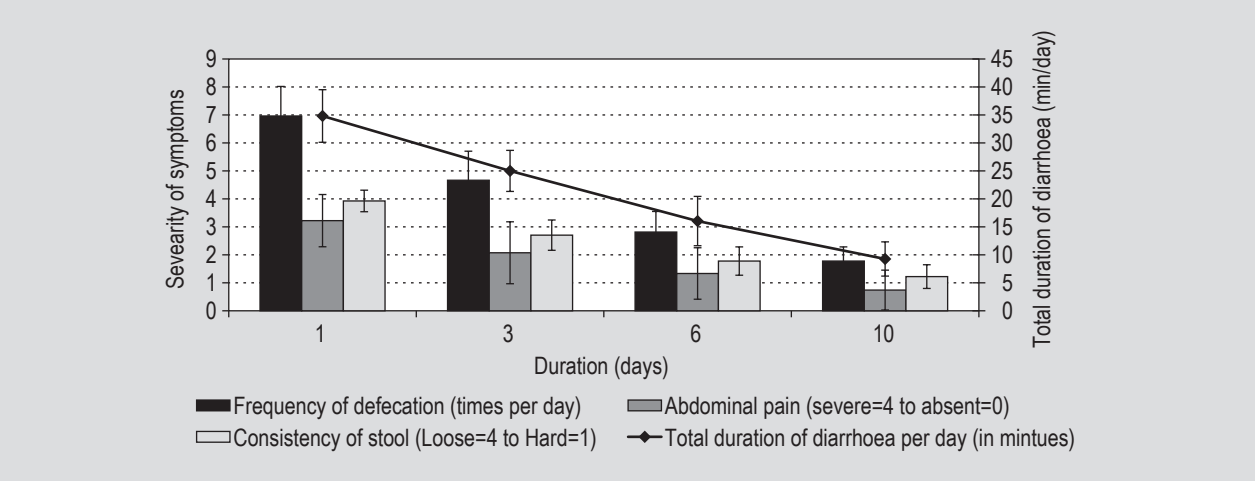

Figure 1. Symptoms of acute diarrhoea during treatment with *Bacillus clausii* strain UBBC-07.

associated with anti-*Heliobacter pylori* drugs (Nista *et al.*, 2004). Similarly, species of *Lactobacillus rhamnosus* GG, *Lactobacillus reuterii, Lactobacillus acidophilus* and *S. boulardii* were also reported to alleviate the symptoms of acute diarrhoea (Szajewska and Mrukowicz, 2001).

The present study demonstrates the effect of *B. clausii* strain UBBC-07 in alleviating acute diarrhoea. All patients reported a significant improvement in various parameters like duration of diarrhoea, frequency of defecation and stool consistency. There are significant changes observed in haematological and biochemical (basophils, neutrophils, monocytes, mean corpuscular haemoglobin and serum glutamic pyruvic transaminase) results. The basophil count was slightly higher, whereas neutrophils, monocytes, mean corpuscular haemoglobin and serum glutamic pyruvic transaminase exhibited a slight decrease in counts or assay. However, these changes were within the normal limits and not considered clinically significant. During the study, none of the patients required rescue medication and none reported any adverse side effects. The treatment of patients with *B. clausii* strain UBBC-07 significantly eliminated the presence of mucus, RBC, WBC, blood and fat in the stool, which demonstrated the efficacy of *B. clausii* strain UBBC-07 in the treatment of acute diarrhoea. Though this study did not explore any mode of action of *B. clausii* UBBC-07, it may be possible that this strain stabilises the gut or possesses antagonistic activity against diarrhoeal pathogens.

The present study was preliminary, as it implied only a small number of subjects and no control group. It can be concluded, however, that the consumption of *B. clausii* strain UBBC-07 has a potential effect in alleviating the symptoms of acute diarrhoea in 10 days.

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