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## Bacillus clausii - The Probiotic of Choice in the Treatment of Diarrhoea

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### Abstract

Probiotics are known to have a role in enhancing digestive health. In this paper, evidence for the efficacy of *Bacillus clausii*, a spore forming probiotic (*viz* clinical studies) in the treatment of diarrhoea, prevention of antibiotic associated diarrhoea and in the prevention of side effects associated with *Helicobacter pylori* is presented. Mechanism of action suggested is through inhibition of pathogens and immunomodulatory effects. *Bacillus clausii* is the probiotic of choice in the treatment of diarrhoea as it has the added advantage of being a spore forming probiotic. It is therefore stable at room temperature and resistant to low pH ensuring that it reaches the small intestines where it can colonize and exert its beneficial effects.

**Keywords:** *Bacillus clausii*; Diarrhea; Probiotic

### Introduction

Probiotics are live microbes, which when administered in adequate amounts confer a health benefit to the host [1]. *Bacillus* species which are spore forming bacteria have been used as probiotics for the last five decades. The advantage of spore forming probiotics over non-spore formers such as *Lactobacillus* spp. is that they are heat stable and can be stored at room temperature without any loss of viability. Spore forming bacteria are also resistant to acidic conditions of the stomach (low pH) and hence can survive the transit to reach the intestine [2,3]. Experimental data suggest that both *Bacillus clausii* (*B. clausii*) spores and cells can adhere to the bowel wall and colonize the mucosa [4]. As *B. clausii* is extremely stable to acidic conditions, the entire dose of ingested bacteria reach the small intestine intact. Three principal reviews have covered the field of *Bacillus* spp. as probiotics [5-7]. The most extensively studies *Bacillus* probiotics are *B. subtilis*, *B. clausii*, *B. cereus*, *B. coagulans* and *B. licheniformis* [8].

### Clinical studies with *Bacillus clausii*

*B. clausii* is a probiotic widely used in Italy since the 1960s for viral diarrhoea in children and for antibiotic related side-effects [9]. We had earlier conducted a study to evaluate the anti-diarrhoeal activity of our *B. clausii* strain UBBC-07 in patients suffering from acute diarrhoea [10].

Diarrhoea is one of the principal causes of morbidity and mortality among adults and children globally [11]. 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 [12]. While viruses are the major causative agent in children, both bacterial and viral pathogens are implicated in adults [13]. 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 [14]. 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 improves stool consistency. Other treatment options include antibiotics, gut motility suppressing agents (e.g., loperamide, codeine), and probiotics [13].

Normal gut microbiota plays an important role in the protection

of the host against gastrointestinal tract diseases [15,16]. 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 [17-19].

In our study, we evaluated the anti-diarrhoeal activity of *B. clausii* strain UBBC 07 in patients suffering from acute diarrhoea. A total of 27 patients (average age of  $35.44 \pm 8.08$  years) with acute diarrhoea were included in a prospective, Phase II clinical study. The inclusion criteria were subjects having  $\geq 3$  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  $2 \times 10^9$  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 indicated that in the probiotic treated group, the mean duration of diarrhoea decreased from  $34.81 \pm 4.69$  to  $9.26 \pm 3.05$  ( $P < 0.0001$ ) min per day, the frequency of defecation also decreased from  $6.96 \pm 1.05$  to  $1.78 \pm 0.50$  ( $P < 0.0001$ ) times per day, abdominal pain decreased from  $3.22 \pm 0.93$  (severe) to  $0.74 \pm 0.71$  (absent) ( $P < 0.0001$ ), and stool consistency improved from  $3.93 \pm 0.38$  (watery) to  $1.22 \pm 0.42$  (soft) ( $P < 0.0001$ ). No significant change in safety parameters were observed during treatment. Our study indicated that *B. clausii* strain UBBC-07 was effective in alleviating the symptoms of diarrhoea without causing any adverse effects.

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Studies conducted by other researchers reaffirm the efficacy and safety of *B. clausii* in reducing the incidence of diarrhoea. We performed and assessed a comprehensive range of relevant literature from PubMed, Medline and other online sources including Google Scholar.

For the PubMed search, the following search terms were initially used (MeSH/All fields) "bacillus" AND "clausii" AND ("diarrhoea OR "diarrhea"). Subsequently, filters like clinical trials and human species were used. The search was restricted only to diarrhoea. Other conditions like upper respiratory tract infections, small intestinal bacterial overgrowth (SIBO) wherein *B. clausii* was used in treatment, were excluded.

*B. clausii* therapy was found to reduce side-effects of anti-Helicobacter pylori treatment in a randomized, double-blind, placebo controlled trial [20]. The effect of *B. clausii* on incidence (primary variable) and severity of antibiotic-associated side-effects during anti-*H. pylori* therapy was studied. One hundred and twenty *H. pylori*-positive patients were randomly screened to receive one of the two treatments (i) a standard 7 days triple therapy with rabeprazole 20 mg b.d., clarithromycin 500 mg b.d. and *B. clausii* t.d.s. (each preparation containing  $2 \times 10^9$ cfu) for 14 days starting from the first day of treatment. (ii) The same 7 days triple therapy and placebo t.d.s. for 14 days starting from the first day of treatment. Side-effects were assessed using a validated questionnaire and were recorded for 4 weeks from the start of therapy. It was found that the incidences of nausea, diarrhoea and epigastric pain in patients treated with *B. clausii* were significantly lower than in placebo group.

*B. clausii* bacteriotherapy was hence found to reduce the incidence of the most common side-effects related to anti-*H. Pylori* antibiotic therapy compared with placebo, i.e., nausea, diarrhoea and epigastric pain.

In a randomized, double-blind, placebo-controlled trial by Maugo, B. M. [21] to determine the effectiveness of *B. clausii* in shortening acute diarrhoeal illness in under-five population with severe dehydration in Kenya, it was observed that when *B. clausii* ( $2 \times 10^9$ CFUs/5 ml vial or placebo, twice daily, for 5 days) was administered to 90 children (age range: 6 months to 5 years) with acute diarrhoea and severe dehydration, the mean duration of diarrhoea in the *B. clausii* group was shorter ( $77.59 \pm 34.10$  hours) than the placebo group ( $86.74 \pm 40.16$  hours). There was a significant decrease in the mean number of diarrhoeal motions on day 3 (*B. clausii* group  $2.74 \pm 1.81$  motions vs. Placebo group  $3.80 \pm 2.7$  motions) and day 4 (*B. clausii* group  $1.45 \pm 1.13$  motions vs. Placebo group  $2.35 \pm 2.19$  motions). There was no significant difference in the duration of stay in hospital between the groups. The conclusion drawn from the study was that in children admitted with acute diarrhoea and severe dehydration, there was a significant decrease in the number of stools as seen on day 3 and 4 of treatment. Although, there was no significant difference in reduction of the duration of diarrhoea and duration of hospital stay in the two groups.

A clinical study on efficacy of *B. clausii* in preventing antibiotic-associated diarrhoea among Filipino infants and children administered *B. clausii* indicated a lower incidence of diarrhoea in the *B. clausii* treated group [22]. The multicenter randomised, open-label, clinical trial, compared two parallel groups of infants and children allocated to either the *B. clausii* probiotic strain or a "no-intervention" control while on antibiotic therapy. The treatment group received a twice daily dose of 1 vial of  $2 \times 10^9$  of *B. clausii* spores within 24 hours of antibiotic initiation and were continued until the last day of antibiotic therapy. The "No Intervention group" was only observed for events.

The duration of treatment was 7 to 21 days. Participants were evaluated daily from day 0 of antibiotic therapy until day 45 post-intervention. Although a prevalence of 31% of children presenting antibiotic-associated diarrhoea was expected in this study, only a total of 10 diarrhoea events (3%) were observed in the included population which was low to reach any conclusion. Seven (7) cases were reported in the "No intervention" group and three (3) cases in the *B. clausii* group but the difference did not reach statistical significance ( $p=0.22$ ). The trend, though not statistically different was towards that of *B. clausii* reducing the incidence of antibiotic associated diarrhoea.

In a separate study, *B. clausii* as an adjuvant therapy in acute childhood diarrhoea was studied in a multicentric trial in India [23,24]. The prospective study included children suffering from acute diarrhoea, at a private tertiary care hospital, India. The children were divided into 2 groups: Group 1 included children on oral rehydration therapy (ORT)+Zinc+*B. clausii* ( $2 \times 10^9$ cfu) and Group 2 comprised of children on ORT+Zinc.

The duration of diarrhoea in *B. clausii* treated group (Group 1) was 22.64 hours as compared to the ORT and Zinc treated group (Group 2) which was 47.05 hours ( $p<0.01$ ).

The frequency of diarrhoea showed improvement within 24 and 60 hours in Group 1 and Group 2 respectively ( $p<0.01$ ). Similarly, the mean duration of hospital stay was 2.78 days in Group 1 and 4.30 days in Group 2. The treatment cost was also significantly reduced. The conclusion from the study was that *B. clausii* reduced the duration; frequency and hospital stay of diarrhoea thereby reducing the treatment and social costs.

Results of all the studies have been summarized in Table 1.

## Conclusion

The clinical benefits observed with probiotic use are mainly attributed to the antimicrobial substances produced by probiotic strains and to their immunomodulatory effects.

*B. clausii* can inhibit the growth of pathogens in the gastrointestinal tract via three distinct mechanisms [21]: colonization of free ecological niches, which are no longer available for the growth of other microorganisms; competition for epithelial cell adhesion, which is particularly relevant for spores in the initial or intermediate germination phase; production of antibiotics and/or enzymes secreted into the intestinal environment, especially peptide antibiotics, which are mainly active on Gram-positive bacteria, but also enzymes that exhibit lytic activity against *Pseudomonas aeruginosa*. Urdaci Maria et al. [25] found *B. clausii* to possess antimicrobial and immunomodulatory activities. *B. clausii* strains were found to release antimicrobial substances in the medium. The release of the antimicrobial substances was observed during stationary growth phase and coincided with sporulation. These substances were active against Gram-positive bacteria, in particular against *Staphylococcus aureus*, *Enterococcus faecium*, and *Clostridium difficile*. The antimicrobial activity was resistant to subtilisin, proteinase K, and chymotrypsin treatment, whereas it was sensitive to pronase treatment. The evaluation of the immunomodulatory properties of *B. clausii* strains was performed in vitro on Swiss and C57 Bl/6j murine cells. The authors [25] demonstrated that *B. clausii* strains, in their vegetative forms, were able to induce NOS II synthetase activity, IFN- $\gamma$  production, and CD4<sup>+</sup>T-cell proliferation.

The conclusions one can draw from the aforementioned studies is that *B. clausii* is an effective probiotic and the probiotic of choice in the

Title	No. of subjects, Age and Age Group (grp)	Duration of Study and dosage	Symptoms	Parameters studied	Results
Efficacy of <i>Bacillus clausii</i> strain UBBC-07 in the treatment of patients suffering from acute diarrhea. Sudha MR, Bhoanagiri S, Asin MK (2013) Beneficial Microbes 4: 211-216.	27 outpatients 15 males and 12 females Grp: 35.44 ± 8.0	10 days <b>One capsule of <i>B. clausii</i> strain JBBC-07 (containing 2 × 10<sup>9</sup> cfu) two times a day for a period of 10 days.</b>	≥ 3 loose stool motions within 24 hours and for more than 7 days.	Duration of diarrhoea, frequency of defecation, abdominal pain and stool consistency were tested on days 1, 3, 6 and 10.	All patients reported a significant improvement in duration of diarrhoea, frequency of defecation and stool consistency by the end of the study.
<i>Bacillus clausii</i> therapy to reduce side-effects of anti- <i>Helicobacter pylori</i> treatment: randomized, double-blind, placebo controlled trial Nista et al (2004) Aliment Pharmacol Ther 20: 1181–1188.	Treatment group- Sixty Outpatients (male/female 33/27) Grp: 46.2 ± 12.83  Placebo- Sixty Outpatient (male/female 25/35). Grp: 43.1 ± 13.36	Treatment group- A triple therapy based on clarithromycin 500 mg b.d., amoxicillin 1 g b.d., rabeprazole 20 mg b.d. for 7 days <b>plus a probiotic preparation, one vial t.d.s. (each vial containing 2 × 10<sup>9</sup> spores of <i>B. clausii</i>, for 14 days, during eradication therapy and 1 week thereafter</b>  Placebo- same treatment without <i>Bacillus clausii</i> in the vial	Affected by gastric <i>H. pylori</i> infection as confirmed by a 13 C-urea breath test.	- <i>Helicobacter pylori</i> eradication was evaluated by means of a 13 C-urea breath 6 weeks after the end of the treatment.  -Validated daily diary for 4 weeks, starting from the first day of the eradicating treatment. The diary contains a questionnaire evaluating onset, intensity and frequency of gastrointestinal side-effects: taste distortion, loss of appetite, nausea, vomiting, epigastric pain, bloating, diarrhoea, constipation and skin rash. The intensity of symptoms was rated using a scale.	There was a significant difference between the <i>B. clausii</i> treated group and placebo in the incidence of nausea, diarrhoea and epigastric pain.  <b>A greater reduction in the risk of diarrhoea was observed in the <i>B. clausii</i> group compared with the placebo group after one and 2 weeks.</b>  The mean intensities and frequencies of nausea and diarrhoea were also significantly lower in the <i>B. clausii</i> group
Effectiveness of <i>Bacillus clausii</i> in reducing duration of illness in acute diarrhoea in children 6-59 months of age admitted with severe dehydration Maugo BM (2012) Diss. University of Nairobi, Kenya, 2012.	Hospitalized patients: Treatment group-90 Placebo group-46  Grp: 6 months to 5 years	<i>B. clausii</i> at 2 × 10 <sup>9</sup> CFUs/5 ml vial or placebo, twice daily, for 5 days.	Acute diarrhoea and WHO criteria of severe dehydration	Duration of diarrhoea -Frequency of diarrhoea -Hospital stay	In children admitted with acute diarrhea and severe dehydration, no significant difference in reducing the duration of diarrhoea and duration of hospital stay in the two groups. However significant decrease in the number of stools was seen on day 3 and 4 of treatment
Efficacy and Safety of <i>Bacillus clausii</i> in Preventing Antibiotic-associated Diarrhea among Filipino Infants and Children: A Multi-center, Randomized, Open-label Clinical Trial R.V Destura (2013) <a href="http://en.sanofi.com/img/content/study/ENTER_L_01125_summary.pdf">http://en.sanofi.com/img/content/study/ENTER_L_01125_summary.pdf</a>	Outpatients: Treatment group=162  Placebo-61  Grp: 6 months to 12 years	Treatment group received a twice daily dose of 1 vial of 2 × 10 <sup>9</sup> of <i>B. clausii</i> spores per orem within 24 hours of antibiotic initiation and were continued until the last day of antibiotic therapy( duration of antibiotic treatment ~ 8 days).	Children on antibiotics for mild to moderate infection of the respiratory, genitourinary or skin and soft tissue.	Occurrence of Diarrhoea	In the <i>B. clausii</i> treated group, lower incidence of diarrhoea.
<i>Bacillus clausii</i> As An Adjuvant Therapy In Acute Childhood Diarrhoea. Keya L et al. (2015) IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) p-ISSN: 2279-0861. Volume 14, Issue 5 Ver. I, PP 74-76	131 Hospitalized children: 83 males, 48 females Gp1-69 Gp2-62  Grp: 6 months to 12 years	Two groups-Group 1 comprised of children who were administered oral rehydration therapy (ORT) with Zinc and <i>Bacillus clausii</i> and Group 2 was treated with ORT and Zinc  Children were administered one mini bottle containing 2 billion spores of <i>Bacillus clausii</i> 12 hourly for 5 days. They were followed up at 6 hours, 12 hours, 24, 36, 48, 60 and 72 hours.	Acute Diarrhoea	-Duration of diarrhoea -Frequency of diarrhoea -Hospital stay -Direct and indirect costs	It significantly reduced the duration and frequency of diarrhoea. In addition, it lowered the hospital stay thereby downscaling the financial burden

**Table 1:** Summary of clinical studies on effect of *Bacillus clausii* treatment on diarrhoea.

treatment of diarrhoea, prevention of antibiotic associated diarrhoea and in the prevention of side effects associated with *Helicobacter pylori* treatment. It has the added advantage of being a spore forming probiotic making it a very resilient, stable probiotic which can be stored at room temperature.

### Conflict of Interest

The authors belong to Unique Biotech Ltd., India which is involved in the manufacturing of probiotics.

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