Beneficial Microbes, 2019; 10(2): 149-154

Wageningen Academic Publishers

Bacillus clausii UBBC-07 reduces severity of diarrhoea in children under 5 years of age: a double blind placebo controlled study

M. Ratna Sudha[1*], N. Jayanthi[1], D.C. Pandey[2] and A.K. Verma[3]

[1]Centre for Research & Development, Unique Biotech Ltd., Plot No. 2, Phase-II, Alexandria Knowledge Park, Hyderabad, Telangana 500078, India; [2]MV Hospital and Research Centre, Department of Pediatrics, Room No 01 314/30, Mirza Mandi Chowk, Lucknow, Uttar Pradesh, India; [3]K.R.M. hospital and Research Centre, Department of Pediatrics, Room No 01 3/92-93,Vijayant Khand Lucknow, Uttar Pradesh, India; sudha@uniquebiotech.com

Received: 11 July 2018 / Accepted: 6 November 2018

RESEARCH ARTICLE

Abstract

Acute diarrhoea is one of the leading causes of mortality in infants and young children. Evidence suggests that probiotics can reduce diarrhoea duration. As the effects of probiotics are strain specific, the effect of *Bacillus clausii* UBBC-07, a safe probiotic strain in the treatment of acute diarrhoea in children was studied. The double blind, randomised, placebo-controlled, parallel group multicentric study was conducted at two outpatient facility sites in Lucknow, India. Children aged six months to five years suffering from acute diarrhoea, were randomly assigned to receive either probiotic (*B. clausii* UBBC-07) spore suspension or placebo suspension twice daily apart from oral rehydration solution (ORS). The duration of treatment was for five days with a follow -up until the 10th day. Outcomes evaluated were duration and frequency of diarrhoea, consistency of stool, fever and vomiting. The duration of diarrhoea was significantly shorter (P<0.05) in patients who received *B. clausii* suspension (75.66±13.23 h) than in placebo treated group (81.6±15.43 h). The average daily number of stools (frequency) was 8.67±3.42 at baseline in treatment group receiving *B. clausii* and 8.53±3.19 in placebo group. By day 4, there was a significant reduction (P<0.01) in frequency of stools in probiotic treated group (3.46±0.66) as compared to placebo group (4.57±1.59). Improvement in stool consistency was also observed in the probiotic treated group as compared to the placebo group. There was no effect on vomiting and duration of fever. *B. clausii* UBBC-07 significantly decreased the duration and frequency of diarrhoea as compared to placebo indicating effectiveness of strain in the treatment of acute diarrhoea in children and could be a safe alternative to antibiotics.

Keywords: *Bacillus clausii* UBBC-07, acute diarrhoea, stool, children

1. Introduction

Diarrhoea is one of the leading causes of death globally and is responsible for 8.6% deaths among children younger than 5 years old (Troeger *et al.*, 2017). This burden is even greater in low-income countries due to poor sanitation, lack of safe water and urgent medical care facilities (Troeger *et al.*, 2017; WHO, 2017). The management of acute diarrhoea is defined as the replacement of lost fluids on sudden onset of three or more loose stools per day by oral rehydration solutions (ORS) (Koletzko and Osterrieder, 2009; Samadi *et al.*, 1983). However, ORS solution does not substantially reduce the severity or duration of diarrhoea (Suh *et al.*, 2010). This is where probiotics have a role as they have been proposed as adjunctive therapy in the treatment of acute, persistent, and antibiotic associated diarrhoea (Allen *et al.*, 2011; Basu *et al.*, 2007; Domingo, 2017; Floch *et al.*, 2011; Szajewska *et al.*, 2006). However, there is no consensus that probiotics are to be a line of treatment for acute diarrhoea and more research is needed to guide the use of a particular probiotic strain and duration of treatment (Guarino, 2008; National Collaborating Centre for Women's and Children's Health, 2009). A number of studies have indicated that probiotics are effective in reducing the severity and duration of acute diarrhoea in children. These include *Lactobacillus rhamnosus* (*Lactobacillus* GG), *Lactobacillus plantarum*,

Bifidobacteria strains, *Enterococcus faecium, Saccharomyces boulardii, Bacillus coagulans,* and *Bacillus clausii* strains and preparations containing a mix of strains (Bastola *et al.*, 2017; Davidson and Butler, 2000; Isolauri, 2003; Saavedra *et al.*, 1994; Szajewska *et al.*, 2006; Tankanow *et al.*, 1990). In spite of the beneficial effects, some of the traditional strains have stability issues in product development which further questions the strains ability in health claims (Forssten *et al.*, 2011; Gueimonde and Sanchez, 2012).

Bacillus is a rod shaped, spore-forming, aerobic or facultative anaerobic, Gram-positive, bacterium. Members of the genus *Bacillus* were recognised as potential probiotics for humans and animals (Elshaghabee *et al.*, 2017). Commercially, the spore producing *Bacillus* probiotics are garnering a lot of interest due to excellent stability and health claims (Ahire *et al.*, 2011; Mingmongkolchai and Panbangred, 2018). Furthermore, data on several clinical trials indicates safety and efficacy of strains in host (Elshaghabee *et al.*, 2017). There are a few studies on spore forming *Bacillus* probiotics in diarrhoea (Sudha and Bhonagiri, 2012; Sudha *et al.*, 2013). Recently, Hatanaka and co-workers (2018) showed the efficacy of *B. subtilis* C-3102 spores for treating loose stools in adults.

B. clausii UBBC-07 (MTCC 5472) has been previously isolated by Unique Biotech Ltd., India and characterised well for probiotic properties, stability during industrial processes, absence of toxin genes, absence of transferable antibiotic traits and safety (Lakshmi *et al.*, 2017; Upadrasta *et al.*, 2016). In a clinical study, we have shown that our novel strain *B. clausii* UBBC-07 can potentially be effective in alleviating the symptoms of diarrhoea without causing any adverse effects in adults (Sudha *et al.*, 2013). In the present study, attempt was made to investigate safety and efficacy of *B. clausii* UBBC-07 spore suspension over placebo in Indian children of age six months to five years suffering from acute diarrhoea.

2. Materials and methods

Study design

This double blind, randomised, placebo-controlled, parallel group multicentric study was conducted at two sites, viz. M.V. Hospital and Research Centre, and K.R.M. Hospital and Research, Lucknow, India (period of September to November 2017). This outpatient study was conducted in compliance with the code of conduct for research involving human volunteers as issued by the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP), Indian Council of Medical Research guidelines (ethical guidelines for biomedical research on human subjects) and the principles of the Declaration of Helsinki. Informed consent forms were approved by the ethical committees of both the hospitals and the trial was registered prospectively with the clinical trial registry – India (CTRI Reg. No: CTRI/2017/08/009543). The study was initiated after obtaining informed consent.

Study population

The total number of patients enrolled was 120 with 119 patients completing the study (Figure 1) (per-protocol population). Of the 119 patients completing the study, 59 patients received probiotic, *B. clausii* UBBC-07 suspension (2×10^9 spores/5 ml and ORS) and 60 patients received placebo suspension and ORS. Placebo suspension consisted of purified water alone. The mean age of the patients was 37.20 (±14.31) months (range of 7-60 months). 72 (60.50%) patients were males and 47(39.50%) patients were females. All subjects in the study were of Indian origin. (Table 1).

Selection criteria

Inclusion criteria were patients of either sex between 6 months to 5 years of age with clinical diagnosis of acute diarrhoea, and who had experienced >3 loose stools in the last 24 h. Other inclusion criteria were subjects should not have had any major illnesses and that parents of the subjects were willing to give written informed consent and willing to follow study procedures.

Exclusion criteria included children with severe malnutrition (weight for height <3 SD of WHO charts), subjects requiring antibiotics during the study period, presence of severe diarrhoea which, in the opinion of the investigator required treatment other than investigational product and ORS, presence of visible blood in the stool, use of probiotics in the last three weeks prior to initiation of the study, use of antibiotics or any antidiarrhoeal medication in the last three weeks prior to initiation of the study, participation in any clinical trial or usage of any investigational product in the past 90 days, known or expected hypersensitivity to any of the active substances or excipients, previous use (within 48 h) of kaolin, pectin, bismuth subsalicylate, racecadotril, loperamide, atropine and other anticholinergic agents.

Randomisation

After having obtained signed, written informed consent from the parents of the study subjects, subjects underwent a screening examination. Subjects complying with inclusion/exclusion criteria were enrolled and randomised by block randomisation to one of the two treatment arms. Based on SAS 9.4 (Cary, NC, USA), randomisation numbers for two treatment groups were generated. Randomisation was conducted using opaque sealed envelopes that were indistinguishable between groups in order for the investigators also to be blinded to the treatment. Each envelope had the assignment of the patient (probiotic or placebo treatment) with 60 envelopes for each group. The

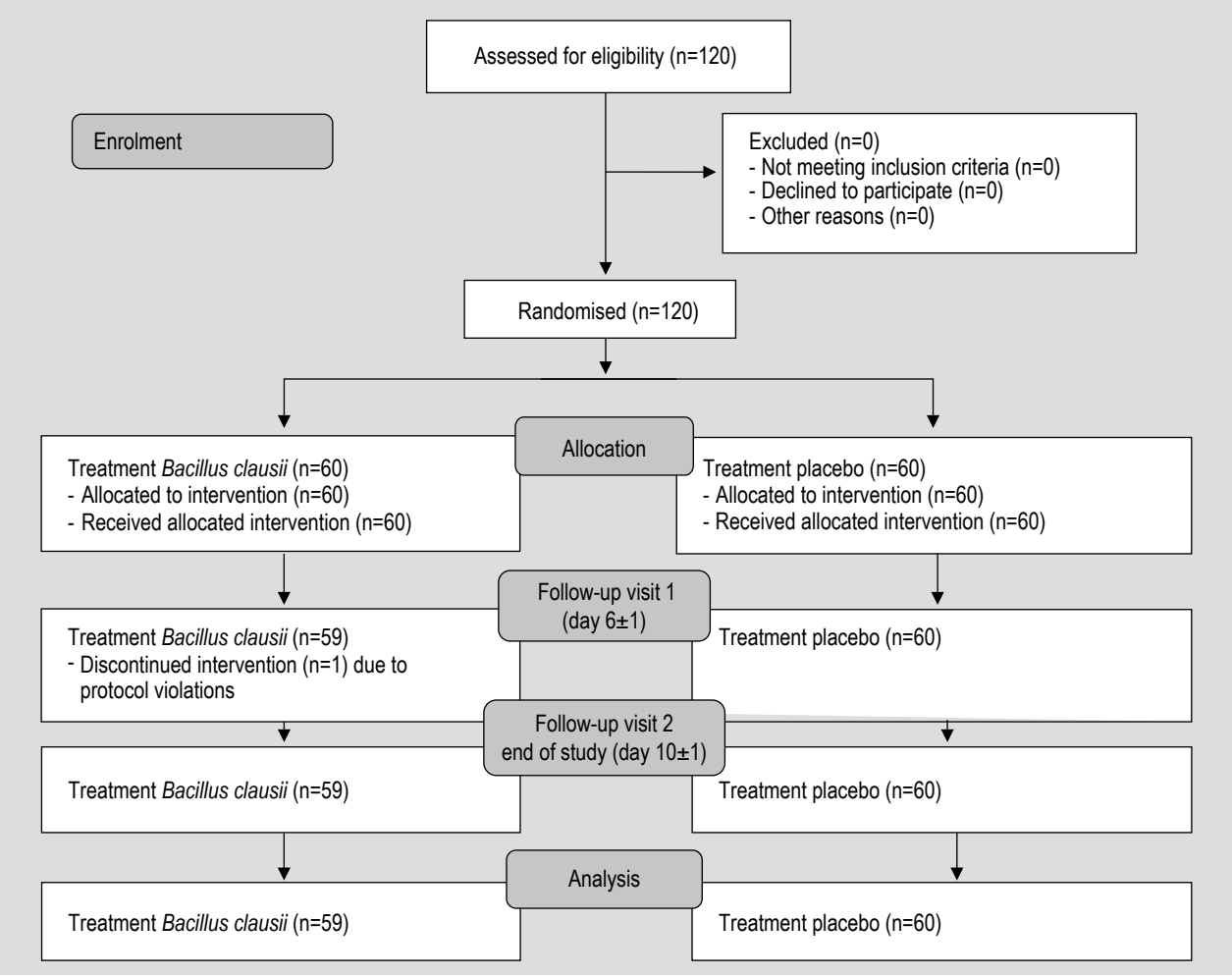

Figure 1. Study flow-chart.

Table 1. Demographic data.[1]

	***Bacillus clausii* UBBC-07 (n=60)**	**Placebo (n=60)**
Mean age (months)	36.63±15.20	37.97±13.42
Range	7-60	7-61
Sex (M:F)	34:26	39:21
Mean weight (kg)	12.87±2.72	12.86±2.33
Range	7.5-17.3	7-17
Mean height (cm)	87.50±12.44	88.62±8.90
Range	40.3-101	68.3-104

[1] Values are mean ± standard deviation.

sealed envelopes were provided to the clinical site. The investigators assigned investigational products to patients based on the randomisation numbers. Both the groups were characteristically similar pertaining to age, sex and weight of the patient.

Study follow-up visits and treatments

The duration of treatment was for a period of five days with a follow up till the 10th day. *B. clausii* UBBC-07 spore or placebo suspension was administered twice daily for a period of 5 days. As this was an outpatient study, three visits were mandatory and recorded: visit 1: Screening and treatment initiation (day 1); visit 2: follow-up after the end of treatment (day 6±1); and visit 3: day 10±1 (end of study).

Efficacy and safety measurement criteria

Decrease in frequency of diarrhoea (where frequency of diarrhoea was estimated as number of motions per day), decrease in the duration of diarrhoea and improvement in the consistency of stool were recorded as primary efficacy measures in the patient diary by the parents. Stool consistency was assessed by Bristol Stool Form scale which categorises the stool into seven types on a scale of 1-7 (hard to loose) 1 and 2 indicating hard stool, 3 and 4 being the ideal stool (especially the latter) and 5, 6 and 7 indicating loose and watery stool.

The secondary efficacy variables were decrease in frequency and duration of vomiting episodes, and decrease in the duration of fever.

Statistical analysis

All efficacy analyses were performed on per-protocol population. Baseline values were compared to post-treatment values using paired *t* test. The two groups were compared for changes from baseline values using one sample *t* test and 95% CI. Measurement data was expressed as means with standard deviation. Categorical data and discrete data was expressed as numbers with percentages (proportions).

3. Results

Primary efficacy

Duration of diarrhoea episodes post-therapy

Duration of diarrhoea was the time in hours from the first to the last abnormal (loose or liquid) stool preceding a normal stool output. The duration of diarrhoea was significantly shorter (P=0.02; Unpaired t test) in patients who received *B. clausii* suspension (75.25±12.96 h) than in patients who received placebo suspension (81.60±15.43 h).

In this study, from day 2 onwards, the number of patients recovering from diarrhoea was more in the *B. clausii* suspension treatment group as compared to placebo group (Figure 2). After 48 h of *B. clausii* suspension treatment, there were 5 (8.47%) children who achieved cessation of diarrhoea compared to 2 (3.33%) children who received placebo. After 72 h, the recovery was markedly higher in the *B. clausii* treated group: 41 (69.49%) children as compared to 35 (58.34%) children who received placebo. After 96 h, there were only 13 (22.04%) children in the probiotic treated group with diarrhoea as compared to 20 (33.33%) children in placebo group.

Number and frequency of diarrhoea episodes (post-therapy)

The average daily number of stools (frequency) was 8.70±3.44 per day at baseline in treatment group receiving *B. clausii* and 8.53±3.19 per day in placebo group (Figure 3). There was decline in average frequency of stools from day 1 to day 5 of treatment in both groups. By day 4 there was a significant reduction (P<0.01) in average frequency of stools in treatment group receiving *B. clausii* (3.46±0.66) in comparison to placebo group (4.57±1.59).

Stool consistency

At baseline, the stool consistency was above 5 (indicating loose, watery motions) in both groups. During the course of treatment, it was observed that by the end of 72 h, 78%

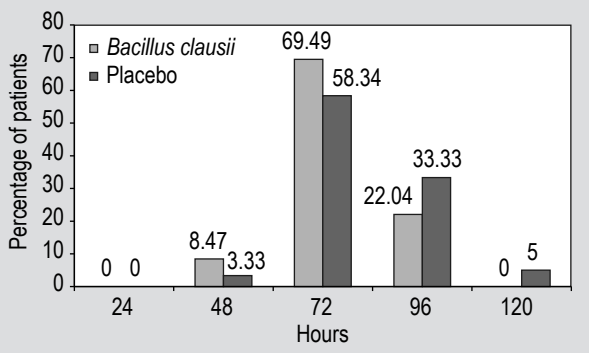

Figure 2. Comparison of time to achieve cessation of diarrhoea during treatment.

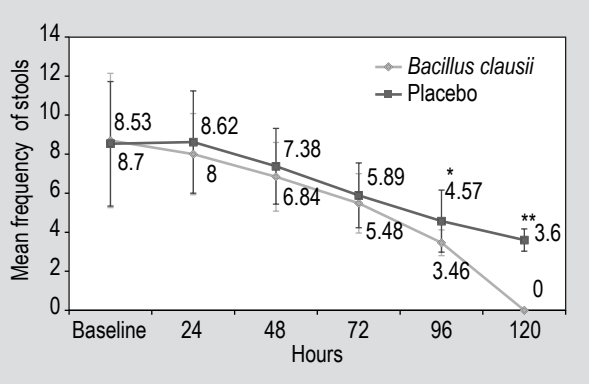

Figure 3. Mean frequency of stools (mean ± standard deviation) from screening till day 5. * *P*<0.01, ** *P*<0.001 between patients taking *Bacillus clausii* and placebo.

(46/59) of the patients had achieved normal consistency in the *B. clausii* treated group compared to 62% (37/60) in the placebo treated group. By the 4th day (96 h), all the patients had achieved normal consistency in the *B. clausii* treated group whereas in the placebo treated group all patients achieved normal consistency only by the 5th day (120 h) (Figure 4).

Secondary efficacy

Among the patients who presented with either vomiting, fever or both conditions in addition to diarrhoea, there was no significant difference in duration of vomiting between the treatment groups as the vomiting subsided in 48 h in both the groups. There was also no significant difference in duration of fever between the two treatment groups. The mean duration of fever was 38.40±13.86 h in children receiving placebo suspension and 37.71±12.17 h in children who received *B. clausii* UBBC-07 suspension.

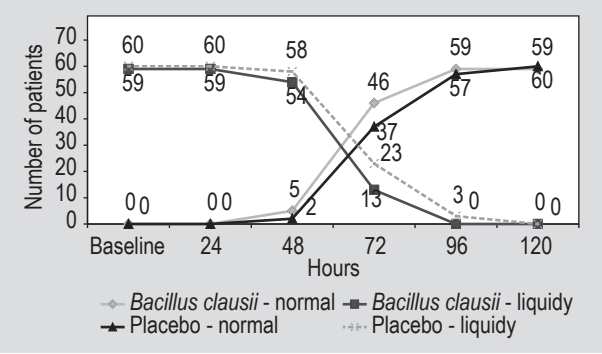

Figure 4. Stool consistency during the course of treatment. Normal = Bristol stool scores 3 and 4; Liquidy = Bristol stool scores 5, 6 and 7.

4. Discussion and conclusions

In the present study, children who received *Bacillus clausii* UBBC-07 had a significantly shorter duration of diarrhoea as compared to the group fed placebo. These results are in agreement with our previous clinical trial of *B. clausii* UBBC-07 on adults with acute diarrhoea (Sudha *et al.*, 2013). The systematic recovery of patients from diarrhoea was higher in probiotic treatment group as compared to placebo indicating strain mediated boost in the recovery. This finding further confirms the anti-diarrhoeagenic effect of *B. clausii* UBBC-07.

The average daily number of stools was significantly reduced in the probiotic treated group as compared to placebo indicating restoration of normal bowel movement. It is interesting to note that both the groups showed decrease in stool frequency, however this decrease was comparatively higher in probiotic treated group. Similarly, at 96 h of *B. clausii* UBBC-07 treatment, all patients in probiotic group showed normal consistency of stool as compared to placebo. No significant difference in duration of fever and vomiting was observed between probiotic and placebo treated groups which is in agreement with the study reported by Canani *et al.* (2007). Our study is in agreement with some of the studies conducted with other strains of *B. clausii*. A systematic review and meta-analysis of randomised controlled trials with *B. clausii* for the treatment of acute diarrhoea in children (Ianiro *et al.*, 2018) concluded that *B. clausii* may represent an effective therapeutic option in acute childhood diarrhoea. For the meta-analysis, six randomised controlled trials (1,298 patients) were selected. Data arising from pooled analysis showed that *B. clausii* significantly reduced the duration of diarrhoea and the duration of hospitalisation compared with control. There was a trend of decreasing stool frequency after *B. clausii* administration compared with the control group. On the other hand, there have also been studies wherein *B.clausii* has been found to be ineffective in the treatment of diarrhoea. The clinical trial reported by Canani *et al.* (2007) showed inability of *B. clausii* to control acute diarrhoea in children. A recent study by Bhat *et al.* (2018) in children suffering from diarrhoea concluded that *B.clausii* did not reduce the duration of diarrhoea though there was a significant reduction in the duration of fever.

In the present investigation we have shown that *B. clausii* UBBC-07 (2×10^9 spores/5 ml suspension; twice daily) significantly reduced the episodes of acute diarrhoea and the associated symptoms of primary efficacy. These results suggest importance of active dose and strain in the treatment of acute diarrhoea (McFarland *et al.*, 2018).

The mechanism of action of *B. clausii* in the treatment of diarrhoea has been attributed to the antimicrobial substances produced and to immunomodulatory effects as well (Maugo, 2012). *B. clausii* can inhibit the growth of pathogens in the gastrointestinal tract via three distinct mechanisms: colonisation 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 *et al.* (2004) demonstrated that *B. clausii* strains 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 evaluation of the immunomodulatory properties of *B. clausii* strains performed *in vitro* on Swiss and C57 Bl/6j murine cells demonstrated that *B. clausii* strains, in their vegetative forms, were able to induce NOS II synthetase activity, IFN-γ production, and CD4+T-cell proliferation.

As per the World Health Organization (WHO) criterion on anti-diarrhoeagenic agents (WHO, 1990), our strain *B. clausii* UBBC-07 significantly decreased the duration and frequency of diarrhoea as compared to placebo indicating effectiveness of strain in the treatment of acute diarrhoea in children. The present study suggests that *B. clausii* UBBC-07, a clinically proven and safe probiotic can be used in the treatment of acute diarrhoea in children.

Conflict of interest

Two of the authors, Ratna Sudha, M. and Jayanthi, N. belong to Unique Biotech Limited which manufactures probiotics including *Bacillus clausii* UBBC-07. They state that the study was conducted independently at the hospital sites with no intervention from them.

References

Ahire, J.J., Patil, K.P., Chaudhari, B.L. and Chincholkar, S.B., 2011. *Bacillus* spp. of human origin: a potential siderophoregenic probiotic bacteria. Applied Biochemistry and Biotechnology 164: 386-400.

Allen, S.J., Martinez, E.G. Gregorio, G.V. and Dans, L.F., 2011. Probiotics for treating acute infectious diarrhoea. Sao Paulo Medical Journal 129: 185-185.

Bastola, R., Bastola, B.S., Gurung, R., Ghimire, J.J. and Shrestha, S.K., 2017. A randomized open label comparative clinical study of a probiotic against a symbiotic in the treatment of acute diarrhoea in children. International Journal of Medical Research and Health Sciences 6: 96-100.

Basu, S., Chatterjee, M., Ganguly, S. and Chandra, P.K., 2007. Effect of *Lactobacillus rhamnosus* GG in persistent diarrhea in Indian children: a randomized controlled trial. Journal of Clinical Gastroenterology 41: 756-760.

Bhat, S., Shreekrishna, G.N. and Savio, C.D., 2018. Efficacy of probiotics in acute diarrhoea in children. International Journal of Contemporary Pediatrics 5: 1646-1650.

Canani, R.B., Cirillo, P., Terrin, G., Cesarano, L., Spagnuolo, M.I., De Vincenzo, A., Albano, F., Passariello, A., De Marco, G., Manguso, F. and Guarino, A., 2007. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. BMJ 335: 340-345.

Davidson, G.P. and Butler, R.N., 2000. Probiotics in pediatric gastrointestinal disorders. Current Opinion in Pediatrics 12: 477-481.

Domingo, J.J.S., 2017. Review of the role of probiotics in gastrointestinal diseases in adults. Gastroenterología y Hepatología 40: 417-429.

Elshaghabee, F.M., Rokana, N., Gulhane, R.D., Sharma, C. and Panwar, H., 2017. *Bacillus* as potential probiotics: status, concerns, and future perspectives. Frontiers in Microbiology 8: 1490.

Floch, M.H., Walker, W.A., Madsen, K., Sanders, M.E., Macfarlane, G.T., Flint, H.J., Dieleman, L.A., Ringel, Y., Guandalini, S., Kelly, C.P. and Brandt, L.J., 2011. Recommendations for probiotic use – 2011 update. Journal of Clinical Gastroenterology 45: S168-S171.

Forssten, S.D., Sindelar, C.W. and Ouwehand, A.C., 2011. Probiotics from an industrial perspective. Anaerobe 17: 410-413.

Guarino, A., 2008. Foreword: ESPGHAN/ESPID evidence-based guidelines for the management of acute gastroenteritis in children in Europe. Journal of Pediatric Gastroenterology and Nutrition 46: 7-8.

Gueimonde, M. and Sanchez, B., 2012. Enhancing probiotic stability in industrial processes. Microbial Ecology in Health and Disease 23: 18562.

Hatanaka, M.K., Suzuki, Y.N., Iio, S., Takara, T., Morita, H., Takimoto, T. and Nakamura, T., 2018. Effect of *Bacillus subtilis* C-3102 on loose stools in healthy volunteers. Beneficial Microbes 9: 357-365.

Ianiro, G., Rizzatti, G., Plomer, M., Lopetuso, L., Scaldaferri, F., Franceschi, F., Cammarota, G. and Gasbarrini, A., 2018. *Bacillus clausii* for the treatment of acute diarrhea in children: a systematic review and meta-analysis of randomized controlled trials. Nutrients 10: 1074.

Isolauri, E., 2003. Probiotics for infectious diarrhea. Gut 52: 436-437.

Koletzko, S. and Osterrieder, S., 2009. Acute infectious diarrhea in children. Deutsches Ärzteblatt International 106(33): 539-548.

Lakshmi, S.G., Jayanthi, N., Saravanan, M. and Ratna, M.S., 2017. Safety assessment of *Bacillus clausii* UBBC-07, a spore forming probiotic. Toxicology Reports 4: 62-71.

Maugo, B.M., 2012. Effectiveness of *Bacillus clausii* in reducing duration of illness in acute diarrhoea in children 6-59 months of age admitted with severe dehydration. PhD-thesis, University of Nairobi, Nairobi, Kenya.

McFarland, L.V., Evans, C.T. and Goldstein, E.J., 2018. Strain-specificity and disease-specificity of probiotic efficacy: a systematic review and meta-analysis. Frontiers in Medicine 5: 124.

Mingmongkolchai, S. and Panbangred, W., 2018. Bacillus probiotics: an alternative to antibiotics for livestock production. Journal of Applied Microbiology 124: 1334-1346.

National Collaborating Centre for Women's and Children's Health UK, 2009. Diarrhoea and vomiting caused by gastroenteritis: diagnosis, assessment and management in children younger than 5 years. Available at: http://tinyurl.com/yabmph24.

Saavedra, J.M., Bauman, N.A., Perman, J.A., Yolken, R.H. and Oung, I., 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. The Lancet 344: 1046-1049.

Samadi, A.R., Islam, R. and Huq, M.I., 1983. Replacement of intravenous therapy by oral rehydration solution in a large treatment centre for diarrhoea with dehydration. Bulletin of the World Health Organization 61: 471-476.

Sudha, M.R., Bhonagiri, S. and Kumar, M.A., 2013. Efficacy of *Bacillus clausii* strain UBBC-07 in the treatment of patients suffering from acute diarrhoea. Beneficial Microbes 4: 211-216.

Sudha, R.M. and Bhonagiri, S., 2012. Efficacy of *Bacillus coagulans* strain Unique IS-2 in the treatment of patients with acute diarrhea. International Journal of Probiotics and Prebiotics 7: 33-38.

Suh, J.S., Hahn, W.H. and Cho, B.S., 2010. Recent advances of oral rehydration therapy (ORT). Electrolytes and Blood Pressure 8: 82-86.

Szajewska, H., Setty, M., Mrukowicz, J. and Guandalini, S., 2006. Probiotics in gastrointestinal diseases in children: hard and not-so-hard evidence of efficacy. Journal of Pediatric Gastroenterology and Nutrition 42: 454-475.

Tankanow, R.M., Ross, M.B., Ertel, I.J., Dickinson, D.G., McCormick, L.S. and Garfinkel, J.F., 1990. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. DICP, the Annals of Pharmacotherapy 24: 382-384.

Troeger, C., Forouzanfar, M., Rao, P.C., Khalil, I., Brown, A., Reiner Jr., R.C., Fullman, N., Thompson, R.L., Abajobir, A., Ahmed, M. and Alemayohu, M.A., 2017. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infectious Diseases 17: 909-948.

Upadrasta, A., Pitta, S. and Madempudi, R.S., 2016. Draft genome sequence of *Bacillus clausii* UBBC-07, a spore-forming probiotic strain. Genome Announcements 4: e00235-16.

Urdaci, M.C., Bressollier, P. and Pinchuk, I., 2004. *Bacillus clausii* probiotic strains: antimicrobial and immunomodulatory activities. Journal of Clinical Gastroenterology 38: S86-S90.

World Health Organisation (WHO), 1990. The rational use of drugs in the management of acute diarrhea in children. WHO, Geneva, Switzerland.

World Health Organisation (WHO), 2017. Diarrhoeal disease fact sheet. Available at: http://tinyurl.com/y75s98s6.