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REVIEW

Bacillus clausii for Gastrointestinal Disorders: A Narrative Literature Review

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ABSTRACT

The gut microbiota is intrinsically linked to human health; disturbances in microbial homeostasis are implicated in both intestinal and extraintestinal disorders. Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," and many commercial preparations comprising a diverse range of species are available. While probiotics have been much researched, better understanding of the probiotic effects and applications of species such as *Bacillus clausii* is warranted. In this narrative literature review, we review the characteristics and mechanisms of action supporting *B. clausii* as a probiotic and discuss the evidence from clinical studies evaluating *B. clausii* probiotics for the management of a variety of gastrointestinal disorders and symptoms in children and adults. Finally, we highlight the challenges of future research and the need for more robust and diverse clinical evidence to guide physicians in the clinical application of probiotics for gastrointestinal disorders and other conditions.

Keywords: *Bacillus clausii*; Diarrhea; Clinical trial; Enterogermina; Gastrointestinal diseases; Gastrointestinal disorders; Microbiota; Dysbiosis

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Key Summary Points

Why carry out this study?

Probiotics are widely used in GI disorders, and properties of spore-forming *Bacillus* strains such as *B. clausii* support their utility in this context

There is a need for better understanding of the probiotic effects and mechanisms of action of *B. clausii* in the treatment of GI disorders

We reviewed literature on the characterization and mechanism of action of *B. clausii*, clinical studies of *B. clausii* in GI disorders, and relevant recommendations and consensus statements

What was learned from this study?

Evidence from clinical studies included in the review suggests that *B. clausii* shows effectiveness in preventing and treating diarrhea in adults and children, including diarrhea resulting from antibiotic treatment

Data from in vitro and in vivo studies support a multi-faceted mechanism of action of *B. clausii*, likely involving immunomodulatory effects and enhancement of mucosal barrier function among other effects

More studies, including well-designed mechanistic studies and randomized clinical trials, are needed to generate high-quality evidence and inform the use of *B. clausii* in GI disorders

INTRODUCTION

The trillions of microorganisms that naturally inhabit the human gastrointestinal (GI) tract and comprise the gut microbiota of an individual reflect a complex and diverse microbial community [1, 2]. The gut microbiota has roles in metabolic, nutritional, protective, structural and neurological functions [2] and as such is intrinsically linked to human health and disease. Disturbances in the normal balance of the gut microbiota, referred to as gut dysbiosis, are implicated in a wide variety of both intestinal and extraintestinal conditions [3, 4]. One approach to aid restoration of gut microbial homeostasis and to re-establish the natural balance of the intestinal ecosystem is administration of exogenous microbes, through foods, supplements or other preparations, that provide beneficial effects for the host.

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," a definition jointly published by the Food and Agriculture Organization of the United Nations and the World Health Organization [5] 2 decades ago and more recently reviewed and upheld by expert consensus [6]. Global interest in probiotics for human health is considerable and expanding, and many products using a diverse range of species are commercially available. These products primarily contain bacterial species of the *Lactobacillus* and *Bifidobacterium* genera [7, 8], which are common members of the endogenous gut microbiota [9], because of their probiotic properties.

Probiotics have been evaluated clinically and the evidence reviewed for a broad spectrum of conditions in adults and children, ranging from GI disorders to allergy, infectious diseases, respiratory conditions, neurological diseases, metabolic conditions and autoimmunity [8, 10–24]. Their use in GI disorders has been the principal focus to date and for which the evidence supporting their health benefits is strongest [25–34].

The GI conditions for which probiotics have been studied include acute gastroenteritis, antibiotic-associated diarrhea, Crohn's disease, celiac disease, inflammatory bowel disease, and *Clostridium difficile* and *Helicobacter pylori* infection [8, 33]. Other GI diseases in which the potential beneficial effects of probiotics have been studied but that are beyond the scope of this review include constipation, necrotizing

enterocolitis, pouchitis and colorectal cancer [27, 33]. The World Gastroenterology Organisation Global Guidelines on probiotics provide evidence-based recommendations for the use of specific strains that are effective for certain GI disorders [30]. The precise mechanisms by which probiotics exert beneficial effects on health are incompletely understood but current evidence suggests multi-faceted mechanisms of action. An overview of proposed mechanisms is provided in Fig. 1, including colonization and restoration of gut microbiota homeostasis; competitive exclusion of pathogenic microbes by secretion of antimicrobials, such as bacteriocins, and competition for nutrients and adherence to the gut epithelia; enhancement of mucin production and mucosal barrier function; secretion of short chain fatty acids; immunomodulatory effects on lymphocytes and cytokines; and modulation of the gut-brain axis [7, 30, 35, 36].

Closely related to the genus *Lactobacillus*, the *Bacillus* genus comprises gram-positive, rod-shaped, spore-forming, aerobic or facultative anaerobic species such as *B. coagulans*, *B. subtilis*, *B. licheniformis*, *B. indicus* and *B. clausii* [7]. Although not traditionally considered a natural inhabitant of the human GI tract, evidence indicates that some *Bacillus* species can colonize the intestinal epithelium, blurring the boundary between gut resident and transient microbiota [37]. Fundamental attributes of the native bacterial microbiota that confer survival are not essential for *Bacillus* species, which show additional characteristics, such as tolerance of acids and bile salts in the hostile environment of the GI tract, that support their use as probiotics [7]. Spore-forming *Bacillus* strains as constituents of foods and pharmaceutical preparations are stable during processing and storage. Several commercially available probiotics include *Bacillus* species, including Enterogermina® (Sanofi), a homogeneous preparation of *B. clausii* (comprising four strains). With prior research focused on *Lactobacillus* and *Bifidobacterium*-containing probiotics, there is a need to better understand the effects and role of *B. clausii* probiotics in human health and disease.

This narrative literature review evaluates *B. clausii* as a probiotic to promote GI health. We first review the properties and mechanisms of action of *B. clausii* that support its credentials as a probiotic. We then discuss evidence from clinical studies in children and adults investigating *B. clausii*-containing probiotics specifically for the management and treatment of GI disorders and symptoms such as diarrhea. Finally, we highlight some of the challenges facing future research and the need for more robust clinical evidence to guide physicians in the use of probiotics for GI disorders and other conditions.

METHODS

Sources and Searches

For this narrative literature review, we initially searched the electronic databases PubMed and Embase and Embase Conference to identify published articles and abstracts reporting studies of *B. clausii*. The terms "*Bacillus clausii*" or "Enterogermina" or derivatives were used in these initial searches. To focus on the setting of GI disorders, a detailed search strategy was employed using general GI disorder terms, microbial terms and gut/microbiome-related terms (Supplementary Tables 1 and 2). No limitations on publication date or article type were specified. All articles and conference abstracts related to oncology/tumor were excluded, and animal studies and duplicates were removed.

Subsequent to the electronic database searches, hand-searching of the literature was conducted to identify studies published prior to the differentiation of *B. clausii* from *B. subtilis* [38] and of non-English language publications. Further relevant articles/abstracts were identified and contributed by individual expert authors of this review.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

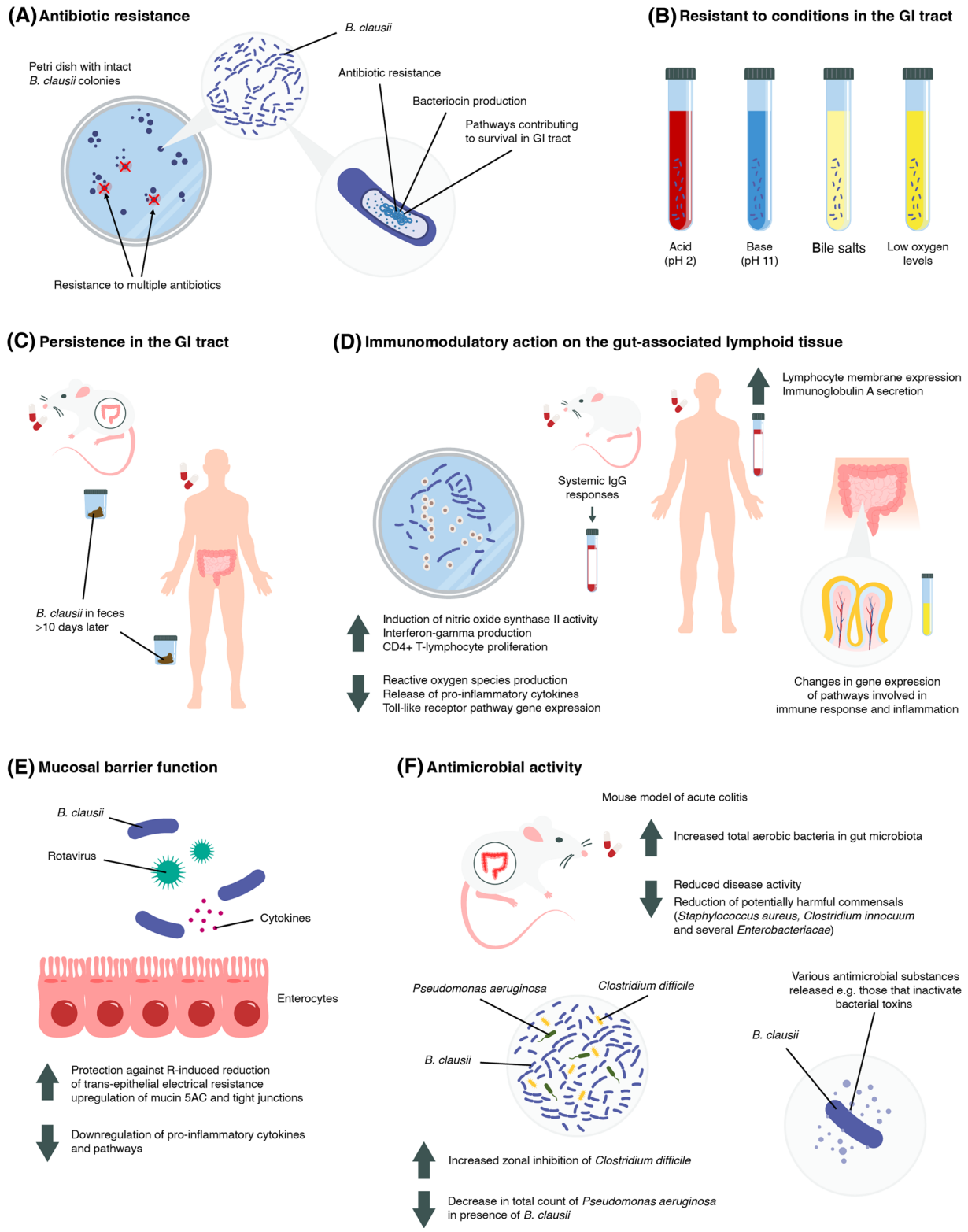

Fig. 1 Potential mechanisms of action of *B. clausii*, based on in vitro and in vivo data. GI, gastrointestinal; IgG, Immunoglobulin G

Data Charting and Analysis

Bibliographic information and associated abstracts from relevant articles were extracted into a spreadsheet. Based on review of the abstracts, articles and conference abstracts were categorized into several key topics, including: (1) Characterization/Mechanism of action; (2) Clinical trials in GI disorders; and (3) Guidelines and Consensus statements. Articles and conference abstracts were identified as to which specific strains of *B. clausii* were reported. Descriptive analysis only of included articles was conducted.

RESULTS

Search Results

The database searches identified a total of 90 articles (including 23 reviews or overview articles) and 13 conference abstracts that were subsequently screened for inclusion in the review. We excluded from the first round of inclusions those publications not directly reporting data for GI disorders of interest or which included animal models, case reports, editorials, expert opinion or narrative reviews related to the use of *B. clausii*. Publications that were subsequently identified from hand searching or at the suggestion of authors were considered for inclusion if they contained data relevant to the overall aims of the review and may otherwise not have met the screening exclusion criteria. Of the 56 articles and abstracts selected following database and hand searching for inclusion in the review, a total of 25 articles/abstracts reported on *B. clausii* characterization and/or mechanism of action (21 reported in vitro data, 2 reported in vivo data and 2 both in vitro and in vivo data), 23 articles/abstracts reported clinical trials, of which 10 were randomized controlled trials, and 7 articles were guidelines or consensus statements.

Characterization and Mechanism of Action of *B. clausii*

As well as discussing identified studies that report *B. clausii*, we also discuss the findings of initial studies investigating the probiotic Enterogermina that were reported as and attributed to strains of *B. subtilis*. Comprehensive molecular characterization has since identified and reclassified the *Bacillus* strains present in Enterogermina as *B. clausii* [38].

In vitro Characterization

Early in vitro characterization of the four *B. clausii* strains in Enterogermina demonstrated broad-spectrum resistance to important antibiotic classes [39], with more recent antibiotic sensitivity profiling defining extensive resistance profiles [40]. The four antibiotic-resistant strains of *B. clausii* comprising Enterogermina are O/C (chloramphenicol), N/R (novobiocin and rifampicin), SIN (neomycin and streptomycin) and T (tetracycline) [39, 41]. Molecular characterization of historic and commercial samples indicates that these constituent *B. clausii* strains show a low level of intraspecific genome diversity, indicating that little variation in the strains has occurred over the past 25 years [38]. Furthermore, a characterization of the composite genome sequence derived from these four *B. clausii* strains has revealed sequences relevant to antibiotic resistance, bacteriocin production, and stress-related and industrially relevant pathways, which together likely contribute to the survival of *B. clausii* within the harsh environment of the GI tract [41]. Studies characterizing the mechanism of antibiotic resistance across the O/C, N/R, SIN and T strains of *B. clausii* have demonstrated the presence of chromosomal sequences conferring resistance to aminoglycosides [42], macrolides, lincosamides and streptogramin B [43], chloramphenicol [44], and rifampicin and streptomycin [40]. Findings describing similar identified genomic features have also been reported following genome profiling of the B106 strain of *B. clausii* [45]. It is noteworthy that resistance of *B. clausii* strains O/C, N/R, SIN and/or T to the antibiotics described above could not be

transferred by conjugation to other bacterial species [42–44].

Different strains of *B. clausii*, including O/C, N/R, SIN and T, several reference strains (DSM 8716^T, DSM 2512, DSM 9783), the UBBC07 strain and two other strains (designated XJ21 and XJ26), have been characterized in vitro for their ability to tolerate conditions mimicking human GI transit [46–49]. Findings from these studies show the ability of *B. clausii* spores to survive, germinate and grow after acid and bile salt challenge and under restricted oxygen levels, with some evidence of variation in responses between the different strains [47]. Separately, strain B106 is likely to exhibit such properties based on the presence of genes encoding proteins related to tolerance of acids, bases and bile salts [45, 50].

Notably, a comparative microbiological and molecular characterization of five commercially available probiotic preparations of *B. clausii* (Tufpro, Ecogro, Enterogermina, Entromax, and Ospor) has shown that some of these comprise mixed bacterial populations; only Enterogermina (O/C, N/R, SIN and T) was found to consist of a homogeneous *B. clausii* population (by two separate analytical approaches) [51].

In vivo Characterization

Persistence within the GI tract of the *B. clausii* strains O/C, N/R, SIN and T has been demonstrated in vivo in a murine model, with declining but detectable levels of the *B. clausii* strains in feces beyond 10 days [46]. These data are supported by the findings of a randomized, open-label, cross-over trial in which healthy volunteers received in turn a single oral dose of *B. clausii* (O/C, N/R, SIN and T) as two formulations, vial and capsule [52]. *Bacillus clausii* was found alive in fecal samples for over 10 days following administration by vial or capsule, at levels higher than the number of spores administered in some subjects and which was most evident for the O/C strain. In fact, another study has demonstrated diverse *Bacillus* species in the feces of untreated healthy subjects, with *B. clausii* reported as the most frequently recovered isolate [53].

Potential Mechanisms of Probiotic Action

The potential mechanisms by which bacterial species harbor a probiotic effect include an immunomodulatory action on the gut-associated lymphoid tissue, the secretion of antimicrobial compounds that suppress pathogen numbers or metabolism of toxins and exclusion of GI pathogens through competitive adherence to the gut wall [35, 46]. Potential probiotic mechanisms for *B. clausii* are summarized in Fig. 1. An immunomodulatory activity of *B. clausii* (O/C, N/R, SIN and T) has been demonstrated in vitro, with exposure to *B. clausii* reported to induce nitric oxide synthase II activity, interferon-gamma production and CD4+ T-lymphocyte proliferation in one study [54]. Furthermore, a mixture of the O/C, N/R, SIN and T strains inhibited reactive oxygen species production and release of pro-inflammatory cytokines and downregulated pro-inflammatory Toll-like receptor pathway gene expression in a cellular model of *Rotavirus* infection [55]. Systemic IgG responses following oral administration of *B. clausii* (O/C, N/R, SIN and T) have been reported in a murine model [46], while increased lymphocyte membrane expression and spontaneous secretion of immunoglobulin A have been demonstrated in healthy adults treated with the same *B. clausii* strains [56]. Treatment with *B. clausii* (strains not specified) was shown to affect the expression of genes involved in the immune response and inflammation in a global analysis of intestinal mucosal samples from a small number of healthy subjects [57].

A recent study examined the potential mechanisms by which *B. clausii* (O/C, N/R, SIN and T) may exert beneficial effects using an in vitro cellular model of *Rotavirus* infection, the most common cause of acute gastroenteritis in young children. Using this approach, Paparo et al. specifically examined biomarkers of mucosal barrier integrity and immune function, demonstrating that *B. clausii* was able to protect enterocytes against *Rotavirus*-induced reduction in trans-epithelial electrical resistance and upregulate mucin 5AC and tight junction proteins, all essential for effective mucosal barrier function. Proinflammatory cytokines and pathways were also downregulated [55].

The in vivo effects of *B. clausii* (O/C, N/R, SIN and T) administration on mild acute colitis and gut microbiota composition have recently been examined in a murine model [58]. Alongside a significant reduction in colitis disease activity versus placebo (days 2–5), *B. clausii* was associated with alterations of the gut microbiota, with a significant increase in total aerobic bacteria and significant reductions of potentially harmful commensals including *Staphylococcus aureus*, *Clostridium innocuum* and several *Enterobacteriaceae*. Furthermore, in vitro co-culture assays showed a significant decrease in the total count of *Pseudomonas aeruginosa* when cultured with *B. clausii* under aerobic conditions. These data support a role for modulation of gut microbiota composition as one possible mechanism through which *B. clausii* exerts beneficial probiotic effects.

The findings of several studies provide evidence that *B. clausii* has antimicrobial activity. In vitro studies have shown that strains of *B. clausii* release antimicrobial substances during the stationary growth phase, coincident with sporulation [54]. Activity against a variety of gram-positive species was observed, including *S. aureus* and *C. difficile*, against which the antimicrobial substance was identified as clausin, a new type A lantibiotic [54, 59] that may interfere with the synthesis of bacterial cell wall components [60]. *Bacillus clausii* antimicrobial activity was shown to be resistant to subtilisin, proteinase K and chymotrypsin, but sensitive to pronase [54]. Further in vitro evaluation of *B. clausii*, specifically strain O/C, has demonstrated inhibition of the cytotoxic effects of *C. difficile* and *Bacillus cereus*, identifying the secreted alkaline serine protease, M-protease, as the substance likely responsible for the observed effects in Caco-2 and Vero cells [61]. Activity against *C. difficile* has also been reported for the UBBC07 strain of *B. clausii* [49]. Multiple strains of *B. clausii*, including O/C, N/R, SIN and T and reference strains DSM 8716^{T}, DSM 9783 and DSM 2512, exhibit inhibitory activity against different genotoxins, restricted to vegetative cells and not evident with spore suspensions [62]. As described above, genome profiling of the B106 strain of *B. clausii* by Kapse et al. [45] identified the presence of numerous genes encoding bacteriocins, supporting an antimicrobial activity of this strain. In contrast to these studies, Duc et al. [46] reported little evidence of bacteriocin production by *B. clausii* (O/C, N/R, SIN and T) following screening assays with 23 indicator strains that included both gram-positive and gram-negative organisms. The reason for the disparity with other studies is not clear, but differences in assay methodology and strains examined may be a factor.

The published literature indicates that *B. clausii* exerts an immunomodulatory effect and possesses antimicrobial activity against a variety of bacterial species, functional properties that support and are likely to be integral to its reported beneficial effects as a probiotic.

Clinical Trials with *B. clausii* in GI Disorders

Below we describe efficacy and safety of *B. clausii* for the treatment of a variety of GI disorders as reported by the studies identified in this review. Details for all these studies are summarized in Supplementary Table 3.

The formulations of *B. clausii* used in these studies, where specified, were mostly vials ($n = 16$) or dry capsules/sachets ($n = 5$). Vials were mostly used in the studies of *B. clausii* in children, whereas, with one exception, capsules were used only in studies involving adults (Supplementary Table 3). The choice of formulations by age group may relate to ease of administration and acceptability of the *B. clausii* preparation by individuals. Although this review was not planned or designed to make cross-study comparisons or meta-analyses, there were no noteworthy differences in results between the formulations used.

Antibiotic-Associated Diarrhea—Prevention

The efficacy of *B. clausii* for reducing diarrhea and other adverse effects associated with antibiotic treatment was assessed in 130 adults (mean age 43 years) with *H. pylori* infection in a phase IIIb randomized, double-blind, placebo-controlled, single-center study by Plomer et al. [63]. Patients were randomized (1:1) to receive one capsule of *B. clausii* (O/C, N/R, SIN and T;

2×10^9 colony-forming units [CFU]) or placebo three times a day (t.d.s.) for 2 weeks. All patients received concomitant antibiotic treatment (clarithromycin 500 mg, amoxicillin 1 g and rabeprazole 20 mg; all twice a day [b.i.d.]) during the first week. Treatment with *B. clausii* compared with placebo was shown to significantly reduce the incidence of diarrhea during week 1 (28% vs 47%, respectively; relative risk [RR] 0.61, 95% confidence interval [CI] 0.37, 0.99; $p = 0.038$), with incidence remaining lower with *B. clausii* in week 2 (RR 0.25, 95% CI 0.07, 0.84; $p = 0.013$). Three adverse effects, none of which were considered serious, were reported in two patients receiving placebo. These data show that supplementation with *B. clausii* was well tolerated and efficacious for reducing episodes of diarrhea associated with antibiotic therapy for *H. pylori*.

Similar findings were reported in an earlier trial by Nista et al. [64]. Adults with *H. pylori* infection ($n = 120$; mean age 44.5 years) received antibiotic therapy (clarithromycin 500 mg, amoxicillin 1 g, and rabeprazole 20 mg; all b.i.d.) for 7 days plus one capsule of *B. clausii* (O/C, N/R, SIN and T; 2×10^9 CFU) or placebo t.d.s. for 14 days in a prospective, randomized, double-blind trial. At week 1, the risk of diarrhea was significantly reduced with *B. clausii* compared with placebo (10% vs 30%; RR 0.33, 95% CI 0.13, 0.85; $p < 0.05$), with a not significant reduction at week 2 (RR 0.50, 95% CI 0.10, 2.61). Significant reductions in the incidence of nausea and epigastric pain were also reported with *B. clausii* compared with placebo. *Bacillus clausii* was also found to be well tolerated, with individual patient's overall assessment of tolerability significantly better than placebo. Thus, treatment with *B. clausii* in patients with *H. pylori* infection reduced the incidence of diarrhea and other side effects of antibiotic treatment with good tolerability.

Some early clinical studies investigated the use of *B. clausii* as a potential preventative measure for diarrhea associated with antibiotic treatment in infants and children. In a study of 35 children (aged 3–24 months) with extraintestinal disease, treatment with antibiotics for respiratory or urinary infections was stopped because of diarrhea in 2/8 subjects receiving only antibiotics, and the number of stools per day increased in most of these subjects; in contrast, the daily number of stools, and their appearance, was normal in children ($n = 11$) who also received *B. clausii* (O/C, N/R, SIN and T; 4×10^9 CFU/day for 5 days) [65]. Similarly, in a study of 93 children (aged 3–14 years) treated with oral antibiotics for bacterial infections, Puddu et al. [66] reported significantly fewer GI problems ($p < 0.01$), including a reduced incidence of diarrhea (1/45 vs 5/48 patients), in children randomized to also receive *B. clausii* (O/C, N/R, SIN and T; 4×10^9 CFU/day for 10 days) versus no additional treatment.

A pooled analysis of three controlled clinical trials, including those described above by Benoni et al. [65] and Puddu et al. [66], has recently examined the effectiveness of *B. clausii* for preventing antibiotic-associated diarrhea in children [67]. Across the three trials, children (age range 3 months to 14 years) presenting with infections of the respiratory tract, genitourinary tract or skin and soft tissue were treated with antibiotics (ampicillin, erythromycin, tetracycline or thiamphenicol) alone or in combination with *B. clausii*. Significant differences in the incidence of diarrhea between treatment groups were not observed in the individual trials. However, in the pooled analysis ($n = 435$ children), treatment with antibiotics in combination with *B. clausii* significantly reduced the incidence of diarrhea compared with antibiotic treatment alone: 4/218 (1.8%) versus 14/217 (6.5%), respectively ($p = 0.017$). While the small sample size and low overall incidence of diarrhea likely explain the lack of statistical significance in the individual trials, the pooled analysis shows that *B. clausii* can help to prevent antibiotic-associated diarrhea in children to a degree similar to that observed in adults.

Antibiotic-Associated Diarrhea—Treatment

Maity and Gupta [68] recently reported findings from a randomized, double-blind, parallel, placebo-controlled trial of *B. clausii* that included both children ($n = 60$, aged 2–10 years) and adolescents and adults ($n = 60$, 11–65 years) with acute infectious diarrhea treated with broad-spectrum antibiotics for the prior 5 days.

Patients were randomized (1:1) to receive *B. clausii* (identified according to new nomenclature as *Alkalihalobacillus clausii*, or *A. clausii*, strain 088AE) as 2×10^9 CFU b.i.d. (children) or t.d.s. (adolescents and adults) or placebo for 7 days, alongside concomitant antibiotic therapy. Compared to placebo, *B. clausii* reduced the total number of unformed stools in both children (24 h: 16.9% vs 0% reduction; 168 h: 99.0% vs 28.5% reduction; $p < 0.0001$) and adolescents and adults (24 h: 57.7% vs 11.0% reduction; 96 h: 98.6% vs 28.3% reduction; $p < 0.0001$). *Bacillus clausii* treatment also resulted in a higher proportion of patients with normal stools and after a shorter duration compared with placebo. Overall, significantly more patients receiving *B. clausii* than placebo achieved complete remission from diarrhea (children: 100% vs 3.0%; RR 30.0, 95% CI 4.3, 100; $p < 0.0001$; adolescents and adults: 73.3% vs 33.3%; RR 2.2, 95% CI 1.27, 3.81; $p < 0.0001$). *Bacillus clausii* was well tolerated by patients, with no reported adverse events (AEs), serious AEs or adverse drug reactions.

In the study by Plomer et al. [63] described above, *B. clausii* was also associated with a significantly higher number of days without diarrhea compared with placebo during week 1 (6.25 vs 5.86; $p = 0.0304$). These findings provide further evidence of the efficacy and safety of *B. clausii* supplementation for managing antibiotic-associated diarrhea.

Diarrhea in Children

The effectiveness of *B. clausii* for treating diarrhea of food or bacterial origin in infancy was investigated in an early, prospective, uncontrolled clinical study by Besana et al. [69]. Thirty infants (aged 20 days to 36 months) presenting with enteritis ($n = 18$), infection-related diarrhea (e.g., bronchitis, cystitis), food intolerance ($n = 3$) or other indication ($n = 3$) were treated with *B. clausii* (O/C, N/R, SIN and T; 1×10^9 CFU) for 5–11 days (1–4 vials/day) and with other as-needed antibiotics. A consistent decline in the number of bowel movements was observed over the first 5 days of treatment, steadily reducing from 3.90 movements/day before treatment to 1.86 movements/day after 5 days; the observed decrease was statistically significant from day 3 onwards ($p < 0.01$). Stool appearance also markedly improved, with only 3.3% of stools being liquid after treatment compared with 76.7% prior to treatment. Treatment with *B. clausii* did not result in clinically significant biochemical findings, and no evidence of side effects or adverse reactions was reported.

A pilot study including 16 infants and children (average age 3 years) suffering from diarrhea of at least 2-day duration reported no statistically significant difference in the clinical course of diarrhea between subjects who received *B. clausii* (O/C, N/R, SIN and T; 3×10^9 CFU/day for 7 days) and those who did not [70]. However, probiotic treatment resulted in a significantly greater eradication of rotavirus and adenovirus infection at follow-up ($p < 0.05$) and was suggestive of a quicker normalization of the total intestinal microflora than seen without *B. clausii*. Similarly, an early study by Benoni et al. [71] reported that the significantly altered bacterial microflora in infants ($n = 9$, aged 3–18 months) with acute diarrhea compared with healthy infants was reversed, at least in part, following treatment with *B. clausii* (O/C, N/R, SIN and T; 4×10^9 CFU/day for 5 days, concomitant with oral rehydration solution).

In a prospective, single-blind, randomized controlled trial, Hamid et al. [72] randomized 300 infants and children (aged 6 months to 6 years) with acute watery diarrhea to standard of care treatment alone or in combination with *B. clausii* (O/C, N/R, SIN and T; 2×10^9 CFU) b.i.d. for 5 days or a multi-strain probiotic once a day for 5 days ($n = 100$ per group). Those treated with the multi-strain probiotic had a significantly shorter mean duration of diarrhea compared with standard treatment alone and treatment with *B. clausii* (2.62 days vs 3.26 days and 3.22 days, respectively; $p = 0.001$). Both the multi-strain probiotic group (days 3–5) and the *B. clausii* group (day 5) showed a significantly lower frequency of diarrhea compared with standard treatment, while both probiotic groups also showed significant improvements in stool consistency by day 4 ($p < 0.05$). In addition, duration of hospital stay was significantly shorter with the multi-strain probiotic group versus both other groups ($p = 0.001$).

Data from this randomized controlled trial indicate that a multi-strain probiotic was effective in reducing the duration and frequency of diarrhea and duration of hospital stay.

Five commercial probiotic preparations, including a homogeneous preparation of *B. clausii* (O/C, N/R, SIN and T; 1×10^9 CFU), given orally b.i.d. for 5 days were evaluated relative to control (rehydration solution) in a randomized, single-blind (investigators blinded) clinical trial including 571 children (aged 3–36 months) with acute diarrhea [73]. Administration of *B. clausii* had no statistically significant effect on duration of diarrhea or daily stool output compared with control. Two preparations, one *Lactobacillus* GG and the other a bacterial mix of four strains, showed significantly lower median duration of diarrhea and daily stool output versus control ($p < 0.001$). Regarding safety, all probiotic preparations were well tolerated, and no AEs were reported. The authors concluded that not all available probiotic preparations are efficacious for the treatment of acute diarrhea in children.

A double-blind, randomized, placebo-controlled, parallel group trial by Sudha et al. [74] evaluated the UBBC07 strain of *B. clausii* (2×10^9 CFU b.i.d. for 5 days) for the treatment of acute diarrhea in children ($n = 119$; aged 6 months to 5 years). Taken alongside oral rehydration solution, *B. clausii* was shown to significantly reduce the duration of diarrhea (75.3 h vs 81.6 h) and daily frequency of stools (day 4: 3.5 vs 4.6; day 5: 0 vs 3.6) compared to placebo (plus rehydration solution). An improvement in stool consistency was also observed in the probiotic group. *Bacillus clausii* had no effect on other outcomes (vomiting and duration of fever). Evidence from this randomized controlled trial indicates that *B. clausii* strain UBBC07 is effective for treating diarrhea in pediatric subjects.

A large-scale, open-label, observational trial by de Castro et al. [75] examined the safety and effectiveness of *B. clausii* (O/C, N/R, SIN and T) as an adjunct to standard therapy in 3178 children (aged 1 month to 6 years) with acute community-acquired diarrhea (viral or antibiotic-associated). *Bacillus clausii*, taken as 2 or 4×10^9 CFU per day for 5–7 days, was well tolerated, with a very low rate of AEs (0.09%), all of which were mild/moderate. Diarrhea was resolved within 3 days of commencing *B. clausii* in 52.6% (1535/2916) of children. *Bacillus clausii* treatment also reduced the mean number of stools per day (from 5.2 stools at baseline to 1.2 stools at study end; $p < 0.001$) and the proportion of children with loose stools (from 81.6 to 9.2%; $p < 0.001$). No treatment-related differences were observed between children with viral versus antibiotic-associated diarrhea. These findings further demonstrate the good safety profile and effectiveness of *B. clausii* for treating acute childhood diarrhea.

B. clausii administered as an adjuvant in children receiving oral rehydration therapy for diarrhea was examined in another prospective, open-label, uncontrolled study by Acevedo et al. [76]. A total of 627 children (aged 6 months to 5 years) with community-acquired diarrhea received *B. clausii* (O/C, N/R, SIN and T; 2×10^9 CFU) b.i.d. for 5 days in combination with rehydration therapy. Over the 5 days, diarrhea resolved in 52% ($n = 311$) of children, with a mean duration of diarrhea of 48.7 h compared with 82.9 h in the total population. Treatment also decreased the mean number of stools per day (5.6 vs 1.8; $p < 0.001$) and the mean occurrence of watery stools (2.4 vs 0.2) from baseline to day 5. Treatment with *B. clausii* was well tolerated, and no treatment-related serious AEs were reported. Pyrexia (2.6%), cough (1.3%) and rhinorrhea (1.1%) were the most common AEs. These data provide further evidence that *B. clausii* can be an effective adjuvant in the management of acute diarrhea in children.

The effectiveness of *B. clausii* in the treatment of childhood diarrhea has also been demonstrated in a systematic review and meta-analysis of six randomized, controlled trials that included 1298 children [77]. In the pooled analysis of these trials, treatment with *B. clausii* (O/C, N/R, SIN and T; any dose) significantly reduced the duration of diarrhea (mean difference: − 9.12 h, 95% CI − 16.49, − 1.75; $p = 0.015$) and duration of hospitalization (mean difference: − 0.85 days, 95% CI − 1.56, − 0.15; $p = 0.017$) compared with control. A

trend of decreasing stool frequency relative to control was also observed following *B. clausii* treatment. A recent systematic review and meta-analysis of randomized controlled trials of probiotics for the treatment of pediatric acute gastroenteritis in India included four studies evaluating *B. clausii* (O/C, N/R, SIN and T; 2–4 × 10^9 CFU/day for 5–6 days) [78]. Like Ianiro et al. [77], pooled analysis of the studies revealed a significant reduction in the duration of diarrhea with *B. clausii* versus control (standardized mean difference: − 1.39 days, 95% CI − 2.74, − 0.04; $p = 0.04$) and a similar but not significant reduction in the length of hospital stay (standardized mean difference: − 1.14 days, 95% CI − 2.91, 0.64; $p = 0.21$). Taken together, these findings suggest *B. clausii* may be an effective therapy for treating acute childhood diarrhea.

Rotavirus-Associated Diarrhea in Children

Two pediatric prospective clinical studies evaluated the effectiveness of *B. clausii* as an adjunctive therapy to reduce the main clinical symptoms of rotavirus infection. In a study of 42 children (aged 1–5 years) with a rotavirus infection who received either standard treatment alone or in combination with *B. clausii*, Smiian et al. [79] reported a significant reduction in the duration of diarrhea (1.09 days), vomiting (0.65 days) and fever (0.61 days) in the probiotic group relative to standard treatment ($p < 0.05$). A similar study by Smiyan et al. [80] also reported that standard treatment plus *B. clausii* (2 × 10^9 CFU/day for up to 8 days) relative to standard treatment alone significantly reduced the duration of diarrhea (1.13 days less; $p < 0.001$), vomiting (0.6 days; $p < 0.001$) and fever (0.52 days; $p < 0.05$) in a cohort of 65 children (aged 6 months to 5 years). Significant changes in immunoglobulin levels during the acute phase were observed before treatment, with normalization of levels during recovery following *B. clausii* treatment but not with standard treatment. Treatment with *B. clausii* was thus shown to have a positive effect on the humoral immune response in children with rotavirus infection.

Diarrhea in Adults

The anti-diarrheal activity of *B. clausii* strain UBBC07 in adults ($n = 27$, average age 35.4 years) with acute diarrhea was evaluated by Sudha et al. [81] in a prospective, phase II, uncontrolled study. Treatment with one capsule of *B. clausii* (2 × 10^9 CFU) b.i.d. for 10 days was effective in significantly reducing the efficacy outcomes of mean duration of diarrhea (from 34.8 to 9.3 min/day), frequency of defecation (from 7.0 to 1.8 times/day), abdominal pain (from 3.2 [severe] to 0.7 [absent]) and improved stool consistency (from 3.9 [watery] to 1.2 [soft]) (all $p < 0.0001$). With no significant changes in safety parameters during treatment, these data indicate that *B. clausii* UBBC07 may represent an effective option for alleviating diarrhea symptoms in adult patients.

A second prospective, phase II, uncontrolled study in adults ($n = 35$, mean age 30.4 years) with acute diarrhea reported similar findings following *B. clausii* treatment (one capsule containing 2 × 10^9 CFU/ml b.i.d. for 7 days), with decreases in mean duration of diarrhea (from 94.2 to 5.8 min/day) and frequency of defecation (from 4.2 to 1.4 times/day) [82]. Stool consistency also improved (from 2.7 [watery] to 1.6 [soft]), while the severity of abdominal pain was reduced. Regarding safety, no significant side effects were observed during the treatment period. These data provide further evidence of the effectiveness of *B. clausii* for treating adults with acute diarrhea.

One early prospective, uncontrolled, preliminary clinical study investigated the effectiveness of *B. clausii* therapy in 18 patients (11 adults, 7 children) with acute or chronic diarrhea due to a variety of GI disorders [83]. All patients were treated for 3 months with *B. clausii* (O/C, N/R, SIN and T; 4 vials of 10^9 CFU/day, with those aged < 5 years receiving half this dose). There were no obvious side effects and no variations of hematochemical indices in any patient. Treatment led to improvements in diarrhea symptoms in 6/7 patients with IgA deficiency or common variety agammaglobulinemia, 3/3 with intestinal neoplasms and 5/5 primary infective diarrhea or subacute infectious diarrhea, but in none of three patients with chronic diarrhea (two with

ulcerative colitis, one with chronic granulomatosis). These findings provided preliminary evidence suggestive of the clinical efficacy of *B. clausii* for treating acute but not chronic diarrhea symptoms associated with GI disorders.

Undiagnosed GI Discomfort

Bacillus clausii has also been evaluated in the management of GI discomfort in the absence of diagnosed GI pathologies. Soman and Swamy [84] conducted a prospective, randomized, double-blind, placebo-controlled, parallel group study in 60 adults (mean age 34.9 years) with symptoms of GI discomfort who were randomized to receive either a multi-strain probiotic blend (containing three *Bacillus* strains, including *B. clausii* [SNZ 1971]) or placebo. The probiotic blend was associated with significant improvements in the Severity of Dyspepsia Assessment scores for burping/belching ($p = 0.025$), bloating ($p = 0.048$), sour taste ($p = 0.025$) and total score ($p = 0.007$) versus placebo (day 30 after treatment start). Subscores for pain ($p = 0.003$), non-pain ($p = 0.04$) and satisfaction ($p = 0.03$) also improved. The multi-strain probiotic blend, which was well tolerated with no AEs, thus appears to be an effective option for treating general symptoms of GI discomfort.

Irritable Bowel Syndrome (IBS)

Using a prospective, pre-test/post-test study design, Acosta-Rodríguez-Bueno et al. [85] investigated *B. clausii* as an adjuvant treatment for pediatric IBS. Following 6 weeks of conventional treatment, children ($n = 15$, aged 6–16 years) then additionally received *B. clausii* (O/C, N/R, SIN and T; 6×10^9 CFU/day) for 6 weeks. Following treatment with *B. clausii*, 93% of children showed an overall improvement in symptoms, compared with 33% of the children after initial conventional treatment alone ($p = 0.04$). Pain intensity (median 6 vs 2; $p = 0.04$) and pain events (median 17 vs 3; $p = 0.03$) were significantly reduced, and the proportion of children with normalization of bowel movements increased (93% vs 13%; $p = 0.01$), following *B. clausii* treatment relative to conventional treatment alone. Cytokine profiling revealed no significant differences between the treatment phases. The findings from this study suggest that adjuvant treatment with *B. clausii* may be beneficial for symptom management in children with diagnosed IBS.

Guidelines and Consensus Statements on Probiotics

Several articles were identified in our literature search that report recommendations and consensus statements on the use of probiotics in GI conditions in adults and children, including those published by/on behalf of the World Gastroenterology Organisation, the European Society for Primary Care Gastroenterology, the European Society for Paediatric Gastroenterology Hepatology and Nutrition, the Mexican Association of Gastroenterology and Malaysian Society of Gastroenterology and Hepatology. Briefly, the overriding position of these articles is that current evidence supports the use of probiotics for GI disorders, with guidance provided on the specific bacterial strains that might be considered in conditions such as antibiotic-associated diarrhea and acute gastroenteritis [30, 86–91]. The article by De Castro et al. [90] presents consensus recommendations from an Asian expert panel specifically on the use of *B. clausii* in acute, chronic and antibiotic-associated diarrhea in children. Specifically, there was sufficient evidence that *B. clausii* may be considered for prevention of antibiotic-associated diarrhea and *C. difficile*-induced diarrhea and as an adjunct treatment during *H. pylori* eradication therapy.

COMMENTARY

Preparations of *B. clausii* are among the many probiotics that are consumed worldwide for their purported health benefits. The clinical studies we identified in this narrative literature review of *B. clausii* probiotics and GI health largely support the beneficial effects of *B. clausii* for managing a variety of GI disorders and their symptoms. From preliminary observations of early clinical studies to recent and robustly designed clinical trials, randomized placebo-

controlled trials and meta-analyses and pooled analyses, the evidence indicates that *B. clausii* is effective for treating and/or preventing antibiotic-associated diarrhea in adults and children [63–68] and acute diarrhea in adults [81–83], with most, but not all [70, 73], studies supporting the effectiveness of *B. clausii* in acute childhood diarrhea [69, 71, 72, 74–80]. Evidence from these studies and clinical use over many decades indicates that *B. clausii* is safe and well tolerated in adults and children [29].

Evidence from in vitro and in vivo studies provides insight into the potential mechanisms through which *B. clausii* is thought to elicit probiotic effects and promote GI health. *Bacillus clausii* has been shown to have immunomodulatory effects on immune cells, cytokine secretion and immunoglobulin levels [46, 54–56], influences mucin production and mucosal barrier function [55], modulates the composition of the gut microbiota [58] and possesses antimicrobial activity [45, 54], including against *C. difficile* [49, 54, 59, 61]. Additionally, *B. clausii* strains show resistance to multiple antibiotic classes [39, 40]. Essentially, strains of *B. clausii* including O/C, N/R, SIN and T tolerate pH and oxygen conditions mimicking human GI transit [46–49], with evidence from animal model and human studies of *B. clausii* in feces over a week after *B. clausii* administration [46, 52]. The reported effects of *B. clausii* and its ability to survive and remain viable during GI transit support its probiotic applications in the treatment of GI and other disorders.

Such characteristics contribute to the reported efficacy of *B. clausii* for treating and preventing diarrhea of varied origin. In addition to supporting data from prospective studies with or without control groups, there is good evidence from randomized controlled trials. Two randomized, double-blind, placebo-controlled trials have demonstrated a significantly lower incidence of diarrhea and related outcomes in adults receiving *B. clausii* (O/C, N/R, SIN and T) concurrently with antibiotic therapy for *H. pylori* eradication [63, 64]. Similar findings with these strains have been reported in children, where *B. clausii* additional to antibiotics resulted in a significantly lower incidence of antibiotic-associated diarrhea in a pooled analysis of three controlled (two randomized) clinical trials [67], while significance was not reached in the individual trials, this likely results from the small sample sizes and low overall incidence of diarrhea. Data from another randomized, double-blind, placebo-controlled trial have demonstrated the efficacy and tolerability of *B. clausii* strain 088AE supplemental to antibiotic therapy for treating children, adolescents and adults with acute infectious diarrhea [68].

Evidence from two randomized controlled trials in acute childhood diarrhea indicates a significantly lower frequency of diarrhea after *B. clausii* treatment (O/C, N/R, SIN and T or UBBC07) versus standard of care [72, 74], although no significant changes in daily stool output were observed in another trial (O/C, N/R, SIN and T) [73]. Moreover, two separate systematic review and meta-analyses of randomized controlled trials demonstrated significantly lower duration of acute diarrhea in children treated with *B. clausii* (O/C, N/R, SIN and T) compared with control [77, 78]. The three studies we identified evaluating *B. clausii* (O/C, N/R, SIN and T or UBBC07) in adults with acute diarrhea were all prospective, uncontrolled trials; even so, improvements relative to baseline were reported for duration of diarrhea, frequency of stools per day and stool consistency [81–83]. Significant improvements in multiple GI outcomes were also identified in a randomized controlled trial in patients with undiagnosed GI discomfort treated with a multi-strain probiotic containing *B. clausii* [84] and in a pre-test/post-test study in patients with IBS who received *B. clausii* (O/C, N/R, SIN and T) with and without conventional therapy [85].

Regarding posology, there was a degree of variation in *B. clausii* treatment regimens between the indications and studies. For patients with antibiotic-associated diarrhea, adults were treated with a daily dose of *B. clausii* of 3–6 $\times$ 10^9 CFU (four studies) while children received 4 $\times$ 10^9 CFU/day (three studies). Treatment regimens lasted at least 5 days, up to 14 days in adults and 10 days in children. Dosing of *B. clausii* for acute childhood diarrhea (12 studies) was 1–4 $\times$ 10^9 CFU/day for 5–11 days, while *B. clausii* was administered as 4 $\times$ 10^9 CFU/day for 7–10 days (two studies)

and 3 months (one study) in adults with diarrhea. The formulations used in these studies were vials in most studies involving children and capsules for those where adults only participated. We found no noticeable differences in outcomes that might be attributable to the formulation. As we discuss later, optimal dosing for individual indications requires further research efforts.

Consistent with prior evidence of the beneficial effects of probiotics for managing a variety of GI disorders, available evidence supports use of *B. clausii* in these indications. The strongest evidence regards the efficacy of *B. clausii* for prevention of antibiotic-induced side-effects, in particular diarrhea in the setting of *H. pylori* eradication [63, 64]. Expert consensus and international guidelines recommend *B. clausii* as adjuvant therapy in this indication [30, 90]. Beyond diarrhea of various origins in adults and children, we found some evidence supporting a role for treatment with *B. clausii* in other GI disorders, including undiagnosed GI discomfort [84] and IBS [85]. Other GI indications for which *B. clausii* may have beneficial effects include bacterial overgrowth of the small intestine [92], functional GI disorders such as functional dyspepsia and pouchitis in inflammatory bowel disease. Indeed, there may be many GI indications for which *B. clausii* and probiotics as a class have beneficial effects, given the proposed mechanisms of action, such as enhancing mucosal barrier function within the GI system [7, 30, 35, 36, 93]. Clearly, clinical studies are needed to better define the specific GI indications for which *B. clausii* is efficacious, more so as the most effective dosing regimens are based on clinical need in each case.

While safety and tolerability were not assessed in all the studies we identified, those that did included studies evaluating diarrhea of various origins in adults and children. Overall, *B. clausii* was reported to be safe and well tolerated in adults [63, 64, 68, 81–83] and in children [68, 69, 73, 75–77]. No serious AEs were reported. Further to this, clinical use for a range of different GI conditions spanning more than 4 decades has shown *B. clausii* to be well tolerated with a very good safety profile in both adults and children [29]. However, it must be noted that a small number of isolated case studies have reported occurrence of bacteremia or septicemia in immunocompetent individuals taking *B. clausii* probiotic preparations, mostly for management of diarrhea. Bacteremia following prolonged administration of *B. clausii* has been observed in several individuals with underlying comorbidities, although no specific risk factors for infection have been identified [94]. An isolated case of *B. clausii* septicemia was reported in a very young (4-month-old) patient with congenital heart disease being treated for diarrhea; *Klebsiella pneumoniae* was also detected in a blood culture from this patient [95]. Administration errors and bacterial translocation have been suggested as potential causes of bacteremia [96], and the presence of GI bleeding may increase the risk of translocation; in one case, an individual with active peptic ulcer disease developed bacteremia after receiving *B. clausii* as part of inpatient treatment for infectious diarrhea [97]. Care should therefore be taken when considering prolonged administration of probiotic preparations to individuals with underlying comorbidities who may be at risk of developing bacteremia.

That commercial probiotic preparations including those containing *B. clausii* are typically provided in sealed containers ready for consumption contributes to their safety by eliminating contamination that might arise during preparatory steps (e.g., mixing with water).

Regarding treatment in children, it is noteworthy that, where assessed by the included studies, children's acceptability of *B. clausii* treatment was high (> 93% good/excellent) [75, 76].

Limitations

The articles discussed in this review were principally identified by searching two electronic databases, PubMed and Embase, for published literature describing studies of *B. clausii*. Potentially relevant literature published in other databases was thus not included, a limitation we readily acknowledge. Also, the output of our search strategy was predicated on the choice of

search terms. While we cannot claim to have identified with absolute certainty every relevant article listed in the two databases, we used relatively broad search terms to assemble a larger body of articles for subsequent screening for relevance. Furthermore, we expanded on the initial searches by manual searching for historic studies (e.g., those citing *B. subtilis* before its reclassification as *B. clausii*), of non-English publications and of the bibliographies of identified articles. A further limitation is that we did not formally assess the quality of included articles, although such an evaluation is more typical of a systemic review rather than the narrative review presented here. Even so, we did observe a relative paucity of evidence from clinical trials evaluating *B. clausii* probiotics, with few or no randomized controlled trials for certain GI indications.

Future Directions

Probiotics are consumed worldwide for their claimed health benefits. Collectively, a broad range of probiotics encompassing different species, strains and preparations have been evaluated clinically for preventing and treating a variety of conditions, notably those of GI origin. Much remains to be established, however. For *B. clausii* and probiotics in general, there is a need for well-designed clinical trials to investigate the efficacy of specific probiotic strains and preparations in different diseases to provide high-quality clinical evidence that can guide physicians in the appropriate selection and use of probiotics for their patients. Such studies will need to better define the optimal dosage and duration of treatment for each individual strain or combinations thereof. Optimizing the clinical application of *B. clausii*-containing probiotics will also require a better understanding of the mechanisms underlying observed health benefits. There is a pressing need, therefore, for mechanistic studies using the latest technologies to explore the characteristics, mechanisms of action and impact on the gut microbiota of *B. clausii* probiotics. This is especially relevant for GI disorders beyond antibiotic-related gut dysbiosis and diarrhea.

Beyond traditional randomized controlled trials, studies using validated models and real-world evidence studies that more closely reflect everyday clinical practice and the real-world habits of patients, given that important factors such as diet and intake of other supplements and medicines (both of which are difficult to control in a trial setting) can affect the microbiota, will further bolster the evidence supporting the probiotic applications of *B. clausii*. Given the varied global epidemiology of GI disorders, impacted by improving hygiene and general health and a decreasing trend in frequency and severity of GI infections, among other factors, future studies will need to determine the efficacy of *B. clausii* probiotics in different geographic regions and across diverse patient populations. This should include more specific subpopulations, such as debilitated patients and those with compromised gut epithelial integrity. With such considerations in mind, comprehensively defining the efficacy and clinical application of individual probiotic strains and preparations remains a significant challenge, one impacted by the changing epidemiology of human disorders and emerging role for gut dysbiosis in disparate conditions.

CONCLUSION

Available evidence from mechanistic, preclinical and clinical studies evaluating a variety of strains and commercial preparations demonstrates the probiotic attributes of *B. clausii* in the setting of GI disorders and their management. In particular, current evidence most strongly supports the use of *B. clausii* probiotic preparations for preventing and treating diarrhea in adults and children, notably that resulting from antibiotic treatment. This review has also brought into focus the relative paucity of high-quality clinical trials evaluating the probiotic applications of *B. clausii*. Well-designed mechanistic studies, clinical trials and real-world evidence, the totality of which is important for evaluating probiotics, are needed to better elucidate its mechanisms of action and to provide high-quality evidence to guide the clinical use

of *B. clausii* in GI disorders and other pathologies.

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