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A comprehensive post-market review of studies on a probiotic product containing *Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011

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Abstract

The probiotic preparation Lacidofil® has been commercially available in Europe, Asia and North America since 1995. This product is a combination of two strains, *Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011. The strains have been evaluated for safety, identity and mechanisms of probiotic action *in vitro*, in animal models and human clinical trials. The strains adhered to human epithelial cells, helped to maintain the barrier function and blocked the adhesion of a number of pathogens, allowing them to be cleared from the intestine. The strains also elicited an anti-inflammatory response by down-regulating IL-1β, IL-8 and TNF-α. In various stress models, the probiotic combination facilitated better coping and outcomes which may be through the maintenance of barrier function and suppressing inflammation. Overall, pre-clinical studies suggest a potential anti-infectious role for the strains and the combination. Clinical studies, primarily in children, have identified Lacidofil as an effective supplement for various gastrointestinal diseases such as antibiotic-associated diarrhoea and acute gastroenteritis. Recent research has also indicated that Lacidofil may be beneficial for individuals with atopic dermatitis or vaginal dysbacteriosis.

Keywords: clinical, antibiotic-associated diarrhoea, infection

1. Background

There are a number of probiotic products on the market. Some are mono-strain products such as *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG or *Lactobacillus plantarum* 299v. Other probiotics are multi-strain products generally having anywhere between two and ten strains. For example, VSL#3 claims four strains of lactobacilli, three strains of bifidobacteria and one strain of *Streptococcus thermophilus*. Multi-strain probiotic products have been shown to be more effective over a broader range of applications than single strain products (Chapman *et al.*, 2011). However, with multi-strain products, it is difficult to understand the role of each microbe present and how they contribute to the overall clinical impact of the mixture. The probiotic Lacidofil® is a two-strain product which has been commercially available since 1995. There have been numerous publications on the individual strains and the whole product *in vitro*, in animal models and in clinical trials. This review examines the totality of the evidence: *in vitro* mechanistic data, animal studies, and human clinical trials.

Lacidofil is composed of *L. rhamnosus* R0011 and *Lactobacillus helveticus* R0052 in a 95:5 ratio. *L. rhamnosus* R0011 was isolated from a dairy starter culture in 1976 and deposited at the Institut Pasteur Collection Nationale de Cultures de Microorganismes (CNCM, Paris, France) as I-1720. The *L. helveticus* R0052 was originally isolated in 1990 from a dairy culture for 'sweet acidophilus milk' and deposited in the Institut Pasteur CNCM as I-1722. Historically, it had been identified as a *Lactobacillus acidophilus* on the basis of its biochemical and metabolic activities, however, subsequent molecular investigation reclassified the identity of this strain as *L. helveticus* (Naser *et al.*, 2006). Because of the various name reclassifications

and alternative strain numbering systems used by various investigators, it can be difficult to follow the documentation that exists for each strain. Table 1 summarises the alternative names that have been used in various publications. Although investigators are strongly encouraged to use the correct strain numbers, often they try to be impartial or blind their samples and therefore prefer to use their own numbering systems.

2. Genome sequencing

The strains in this product were sequenced at Genome Québec (Montréal, Canada) using pyrosequencing (454 Life Sciences, Branford, CT, USA) and the genomes have been uploaded to GenBank. *L. rhamnosus* R0011 Bioproject Genome ID: 51799 (accession: PRJNA51799). *L. helveticus* R0052 accession: AEIR01000000. No antibiotic resistance genes or other genes of concern were observed in the genome of either strain. A 6.4 kb cryptic plasmid was identified in *L. helveticus* R0052. This unique plasmid has been sequenced (GenBank accession: FJ851149) and its minimal origin of replication was identified (Hagen *et al.,* 2010).

3. Toxicity studies

Toxicity studies, completed by Evic-Tox (Blanquefort, France) in male and non-pregnant female specific pathogen-free Sprague-Dawley albino rats (Charles River Laboratories, L'Arbresle, France), have been previously described (Tompkins *et al.,* 2008), and no toxicity was observed (Evic-Tox report to the corresponding author: study reference TL 053 / 05-0678 and TL 054 / 05-067).

4. Pharmacovigilance and safety monitoring

Lacidofil has been sold as a dietary supplement or a pharmaceutical product as a powder in capsules or in sachet format in a number of countries since 1995. Since the launch of the product in 1995, there have been less than ten non-serious events, in the form of either diarrhoea or rashes, reported by health authorities or directly by the consumer (personal communication to the authors from the Market Authorisation Holder). In the last five years there have only been four reports (two diarrhoea and two skin rashes), three of which occurred in children; the age of the fourth person was not provided. In all cases, probiotic therapy was stopped and the condition cleared without requiring additional treatment. It is not clear whether the reactions were a result of the Lacidofil intervention or an extenuating condition associated with the pre-existing malady. In addition, the potential role of the excipient used in the preparation of the product, rather than the probiotic bacteria, as a trigger of these events cannot be discounted. As per current regulations, the Market Authorisation Holder is obliged to regularly submit Periodic Safety Update Reports to the various government health agencies.

5. *In vitro* studies pertaining to potentially probiotic mechanisms of action

The lactobacilli in Lacidofil have been characterised *in vitro* for their activities which may impact the host. Viable *L. rhamnosus* R0011 has been shown to produce exopolysaccharides; adhere to human intestinal epithelial cells (IEC); maintain the barrier function as shown by trans-epithelial electrical resistance; and down-regulate pro-inflammatory pathways (Table 2). With human colonic adenocarcinoma cells, HT-29, the strain was able to reduce the association, invasion and translocation of pathogenic *Campylobacter jejuni* (Alemka *et al.,* 2010); however, this reduction was not seen with another human colonic adenocarcinoma cell-line, T84 (Wine *et al.,* 2009). This suggests that there may be some specificity for interaction of R0011 with human cells.

Human HT-29 or KATO III (gastric adenocarcinoma cells) stimulated with lipopolysaccharide derived from *Escherichia coli* yield a strong pro-inflammatory response as characterised by increased levels of interleukin-8 (IL-

Table 1. Alternate names used in the literature to describe the strains found in Lacidofil®.

Strain	Alternate name	Reference
R0011	LB24	Champagne and Gardner, 2008
	R	Dupont *et al.,* 2000; Pham *et al.,* 2000; Van Calsteren *et al.,* 2002; Provencher *et al.,* 2003; Peant *et al.,* 2005
	R-11	Botes *et al.,* 2008
	R-011	Roy and Ward, 2004
R0052	I-1722	Possemiers *et al.,* 2010
	K1	Podlesny *et al.,* 2011
	K300	Atassi *et al.,* 2006
	Rosell-52	Diop *et al.,* 2008
	Lactobacillus acidophilus YS or RO-52	Easo *et al.,* 2002

Table 2. *In vitro* studies with the strains in Lacidofil®.

Strain	Action	Observation	Reference
R0011	exopolysaccharide production	production of specific exopolysaccharides which may have role in reduction of oxidative stress, as examined in Sengul *et al.,* 2011	Dupont *et al.,* 2000; Peant *et al.,* 2005; Pham *et al.,* 2000; Provencher *et al.,* 2003; Van Calsteren *et al.,* 2002
	adhesion and barrier function	adhesion and prevention of loss of trans-epithelial electrical resistance in *Escherichia coli* challenged cells. Improved tight junctions and barrier effect	Sherman *et al.,* 2005
		upregulation of mucin gene (muc3) in the jejunum and ileum	Dystra *et al.,* 2011
	pathogen inhibition	prevent the association, invasion and translocation of *Campylobacter jejuni*	Alemka *et al.,* 2010; Wine *et al.,* 2009
	immune modulation	downregulation of pro-inflammatory cytokines and interleukins	Fiander *et al.,* 2005; Wallace *et al.,* 2003; Wood *et al.,* 2007
R0052	adhesion and barrier function	adhesion to human IEC	Sherman *et al.,* 2005
	pathogen inhibition	*Salmonella typhimurium* and *E. coli*: ↓ pathogen growth; ↓ pathogen adhesion; ↓ pathogen internalisation	Atassi *et al.,* 2006
		E. coli: ↓ *E. coli* adhesion; maintain tight junctions of IEC; block cytoskeletal rearrangement by *E. coli*	Jandu *et al.,* 2009; Johnson-Henry *et al.,* 2007; Sherman *et al.,* 2005
		production of a unique S-layer protein which prevents the adhesion of *E. coli* to IEC and improved barrier function	
		C. jejuni: reduced the adhesion and virulence	Alemka *et al.,* 2010
		Staphylococcus aureus: production of bacteriocin-like inhibitory substances to reduce the viability	Sadowska *et al.,* 2010
	immune modulation	downregulation of pro-inflammatory cytokines and interleukins and induction of antibody class switching: ↑ IgM; ↑ IgG; ↑ splenocyte proliferation; ↓ IL-8; ↓ Rantes;	Easo *et al.,* 2002; Wallace *et al.,*2003

8) (Wood *et al.,* 2007). In these cell systems, R0011 was able to significantly reduce the levels of IL-8. Wood *et al.* (2007) demonstrated that vasoactive intestinal peptide (VIP) was able to abrogate the down-regulatory effect of the *L. rhamnosus* R0011. VIP is known to induce protein kinase A and elevate cAMP. Direct activation of protein kinase A and C by forskolin and phorbol myristate acetate, respectively, impeded the ability of R0011 to block IL-8 production in LPS-stimulated cells. This outcome suggested that high levels of induction of protein kinases render human intestinal epithelial cells refractory to the modulatory effects of the *L. rhamnosus* R0011 (Wood *et al.,* 2007).

Cell cultures of Caco-2 subclone C2BB31 or HT-29 intestinal epithelial cells, when induced by IL-1β, have significantly elevated levels of prostaglandin E2 and E2α. Milk fermented by R0011 was able to eliminate the prostaglandin E2 and prostaglandin E2α response (Fiander *et al.,* 2005). In this case, the fermented milk components were shown to have more impact than the microbe itself, but the action of fermentation by the viable R0011 was necessary as the heat-killed form had no impact. Fiander *et al.* (2005) further showed that the suppressive effect of the R0011 ferment could be blocked by opioid receptor antagonist naltrexone, suggesting that the R0011 may be producing opioid-like peptides or other endocrine-like factors during fermentation that could directly influence the human IEC, as hypothesised by Lyte (2011). The purified exopolysaccharides, the culture supernatant and the heat-inactivated version did not have any impact on the adhesion of pathogenic *E. coli* (Johnson-Henry *et al.,* 2007; Sherman *et al.,* 2005), reinforcing the importance of having the live, intact bacteria to have the full probiotic benefit.

The requirement for an intact and viable R0011 was also demonstrated by Dykstra *et al.* (2011) who showed that mucin expression (muc3) was augmented in the jejunum and ileum of rodents only when these parameters were maintained. Overall, the evidence suggests that live *L. rhamnosus* R0011 acts by maintaining the intestinal permeability and the protective mucosal layer and by downregulating the proinflammatory pathways in models where these pathways have been upregulated. The exact mechanism by which this is achieved has not

been elucidated, but it does not appear to be related to its adhesive capacity nor its production of exopolysaccharide (Johnson-Henry *et al.,* 2007; Sherman *et al.,* 2005). Its mechanism of action may be related to the ability of *L. rhamnosus* to produce specific proteins and peptides, as such activity has been demonstrated previously for *L. rhamnosus* GG (Yan *et al.,* 2007). Homologous genes encoding p40 and p75 proteins described by Yan *et al.* (2007) have been identified in the genome of R0011. The exopolysaccharides produced by R0011 may have a role in reducing intestinal damage due to oxidative stress (Sengul *et al.,* 2011), but this has not been investigated.

Viable *L. helveticus* R0052 has been demonstrated to act in many of the same conditions as *L. rhamnosus* R0011, but probably through different mechanisms. Foremost, the R0052 was highly adherent to human IEC and able to block the association, adhesion, invasion and translocation of *E. coli, Salmonella typhimurium* and *C. jejuni* (Table 2). This adherent property of the R0052 has been linked to its unique surface layer protein (SlpA) (Johnson-Henry *et al.,* 2007). In terms of immune modulation, in addition to down regulating pro-inflammatory cytokines and interleukins, R0052 can induce antibody class switching and can increase anti-pathogen antibodies (Easo *et al.,* 2002). All of these mechanisms of action appear to require the presence and involvement of specific SlpA on the surface of the strain, as the functionality of the SlpA in a *S. typhimurium* FP1 model of infection has been subsequently confirmed in another *L. helveticus* strain M92 (Beganovic *et al.*, 2011). Furthermore, R0052 may be able to inhibit the growth and outright kill some pathogens in co-culture (Atassi *et al.,* 2006). The mechanism by which this can occur has not been determined, but it may simply be an effect of rapid acidification of the media with acetate, and lactic acids. However, recent investigations by Sadowska *et al.* (2010) suggest the presence of a bacteriocin-like substance that is active against *Staphylococcus aureus*. Examination of the R0052 genome shows the presence an open reading frame (locus tag: R0052_0577; nt position: 555195-556172) potentially encoding a helveticin J homologue (89% identity to the helveticin J homologue in *L. acidophilus* NCFM) and an additional novel helveticin-like protein (locus tag: R0052_0107; nt position: 98614-99591). The expression of these genes in R0052 was confirmed by reverse-transcriptase PCR (T.A. Tompkins, unpublished results), but their role in antibacterial activity has not been confirmed.

6. Studies in rodent models of infection

Animal studies with Lacidofil can be broadly classified into two main categories, those dealing with infection and those dealing with stress (Table 3). The infectious models have focussed on three pathogens: *Citrobacter rodentium* as a rodent model for enterohaemorrhagic *E. coli* (EHEC) such as strain O157:H7; *Helicobacter pylori* models of gastric ulceration; and *Candida albicans* infections following gastric ulceration or ulcerative colitis.

Infection of adult mice with *C. rodentium* will cause them to develop attaching-effacing lesions, mucosal inflammation and epithelial cell hyperplasia (Higgins *et al.,* 1999), however, it is generally self-limiting and life-long immunity is maintained after recovery (Borenshtein *et al.,* 2008). Infant mice are more susceptible to this infectious microbe and will develop serious adverse effects and eventually die (Gareau *et al.,* 2010). Pre-treating adult mice with Lacidofil prior to *C. rodentium* administration significantly decreased the load of the pathogen in the colon, mucosal inflammation, epithelial cell hyperplasia and colonic apoptosis. However, it did not impact the interferon-gamma production by the splenocytes (Johnson-Henry *et al.,* 2005). These authors suggested that there were inhibitory effects of the probiotic on the pathogen via secreted substances. In the neonatal mouse model the *C. rodentium* caused severe diarrhoea, weight loss and eventually death (Gareau *et al.,* 2010). Daily pre-treatment with the probiotic mixture prevented weight loss and death. As in the adult system, the probiotics reduced hyperplasia and *C. rodentium*-induced mucosal barrier dysfunction. They went on to show that the effects of the probiotic could be attributed to its ability to modulate the hypothalamus-pituitary-adrenal (HPA)-axis

Table 3. Studies in rodent models.

Indication	Study	Reference
Infectious models	*Candida albicans* infection, inflammation and ulcer healing in a rat model	Brzozowski *et al.,* 2005; Zwolinska-Wcislo *et al.,* 2007, 2009
	Helicobacter pylori models	Brzozowski *et al.,* 2006; Johnson-Henry *et al.,* 2004; Verdu *et al.,* 2008
	Citrobacter rodentium infection in mice as analogue for *Escherichia coli*	Gareau *et al.,* 2010a, 2011; Johnson-Henry *et al.,* 2005
Stress models	post-infectious stress: behaviour modification	Gareau *et al.,* 2010b; Verdu *et al.,* 2008
	psychological stress: water avoidance	Zareie *et al.,* 2006
	psychological stress: maternal separation	Gareau *et al.,* 2007

as serum corticosterone levels remained low in probiotic-treated animals. In addition, using either B-cell (JH -/-) or T-cell (rag1 -/-) deficient mice, they showed that the protective properties of the probiotic were mediated via T-cells but not B-cells.

The studies of *H. pylori* infection have taken various forms. Johnson-Henry *et al.* (2004) demonstrated that pre-treatment with Lacidofil before *H. pylori* challenge decreased the colonisation of the gastric mucosa by the pathogen and reduced the gastric inflammation in mice. Unlike the *Citrobacter* situation, the probiotic did not prevent pathogen-induced epithelial cell apoptosis, however, the difference may be associated to the site of apoptosis, as *H. pylori* infection occurs in the gastric epithelial cells while *C. rodentium* occurs in the colon.

H. pylori infection in Mongolian gerbils is an established experimental model of gastric carcinogenesis that mimics the development of gastric ulcers and gastric cancer in *H. pylori*-positive patients (Brzozowski *et al.*, 2006). To ascertain the impact of the probiotic product in this model, it was given to Mongolian gerbils, post-infection with *H. pylori*, and the outcomes were compared to gerbils given vehicle only or conventional triple eradication therapy (Brzozowski *et al.,* 2006). Both conventional therapy and Lacidofil maintained gastric acid, plasma gastrin and luminal somatostatin levels. Mucosal inflammation, gastric lesions, hyperplasia and apoptotic body formation were completely eliminated by the conventional triple therapy and significantly attenuated by the probiotic alone. The *H. pylori* infected animals showed significantly increased levels of COX-2 and BAX while Bcl-2 was significantly repressed; both conventional triple therapy and Lacidofil given post-infection attenuated or eliminated these responses. Verdu *et al.* (2008) demonstrated that BALB/c mice with chronic *H. pylori* infection had persistent behavioural and physiological changes even after the pathogen had been eradicated. These changes, such as delayed gastric emptying, increased intestinal permeability, and increased gastric $CD3^+$ cell counts, led to altered feeding behaviour. The mice in which the *H. pylori* had been eradicated were given either the probiotic or a placebo. Those that received Lacidofil had accelerated recovery of paracellular permeability, but the effect was modest compared with the placebo group. However, the probiotic did normalise gastric emptying, and improved the $CD3^+$ cell counts. The feeding patterns were also normalised in the probiotic group, but not the placebo group. Thus, in this case, the changes in gastric emptying and feeding behaviour did not appear to be mediated by an improvement in small intestine permeability.

C. albicans infections can slow healing in individuals with chronic inflammatory diseases or other intestinal disorders (Brzozowski *et al.,* 2005). Elimination of fungal infections can be difficult as the antifungal therapeutics may have adverse side effects (De Rosa *et al.,* 2009). The impact of Lacidofil on various models of ulceration has been investigated. Brzozowski *et al.* (2005) induced gastric ulceration in male Wistar rats by applying acetic acid directly to the anterior serosal surface of the stomach at the antro-oxyntic border. Rats were randomised and inoculated with *C. albicans* or saline. The rats were then treated with ranitidine, an anti-secretory agent, or the non-steroidal anti-inflammatory drug acetylsalicylic acid (ASA) with or without Lacidofil and recovery was monitored. In saline inoculated rats, the ulcers disappeared by day 25. *Candida* inoculation caused persistent ulceration, a fall in gastric blood flow and gastric acid output, and a rise in plasmic gastrin. Furthermore, inflammatory immune factors (IL-1β, TNF-α, EGF and TGF-α) were upregulated in the *Candida*-infected rats. The ranitidine and ASA treatments delayed the healing even further, but Lacidofil reversed all the measured parameters to resemble uninfected, saline-inoculated rats. Lacidofil reduced the *Candida* colonisation and suppressed the pro-inflammatory cytokine levels (IL-1β and TNF-α) thereby accelerating healing. The authors conclude that the probiotic was effective in the treatment of gastric ulceration (Brzozowski *et al.*, 2005; Zwolinska-Wcislo *et al.*, 2006).

A similar study was done in a rat model of colonic ulcerative colitis (Zwolinska-Wcisko *et al.*, 2007, 2009). In this study, trinitrobenzene sulfonic acid (TBNS) was rectally administered to male Wistar rats to induce colonic ulceration. The ulcerated rats were inoculated with *Candida* or with saline. These groups then received no treatment, Lacidofil or fluconazole. The TNBS ulceration caused an increase in colonic weight due to inflammation, a decrease in colonic blood flow, in increase in myeloperoxidase (MPO) levels (as a marker of colonic neutrophil infiltration). Groups that were inoculated with *Candida* showed delayed healing and elevated levels of plasma IL-1β and TNF-α. Administration of either fluconazole or Lacidofil to the *Candida* infected rats significantly decreased the weight of the colon segments, the MPO activity and the IL-1β and TNF-α levels. There was no significant difference between the fluconazole and Lacidofil treatments, both were equally effective in minimising the impact of *Candida*.

Overall the various pathogen challenge models show that the Lacidofil acts to reduce the pathogen load and to modulate the pro-inflammatory responses.

7. Studies in rodent models of stress

The impact of Lacidofil on the brain-gut axis has been evaluated in four rodent models: two post-infection models and two psychological stress models.

As described earlier, Verdu *et al.* (2008) showed that *H. pylori* infection induced a persistent alteration of feeding

behaviour such that the mice ate frequently but took in only small portions. Post-eradication treatment with Lacidofil restored normal nocturnal feeding behaviour. No mechanism by which this could occur was presented; however, the decreased inflammatory response was seen as a contributing factor.

The other post-infection model presented by Gareau *et al.* (2010) demonstrated that adult C57BL/6 mice and germ-free Swiss-Webster mice showed impaired memory when inoculated with a non-invasive pathogen, *C. rodentium*, and exposed to water avoidance stress. The germ-free mice showed memory loss upon infection even without applied stress. The behaviour of these animals was evaluated using object recognition and T-maze testing. Anxiety was evaluated by light preference using a light/dark box, however, no change in anxiety was observed. The changes in memory persisted after the clearance of the *C. rodentium* and resolution of intestinal injury. In those mice treated with the probiotic, the colonic cell hyperplasia was restored and serum corticosterone and INF-γ levels were ameliorated, but not the TNF-α level. In addition, exposure to the probiotic prevented a drop in expression of cFos and brain-derived neutrophilic factor (BDNF) in the CA1 hippocampus. Pre-treatment with Lacidofil prevented the stress-induced memory deficits. The authors conclude that the memory loss following infection and water avoidance stress was mediated, in part, through the reduction of the BDNF in the hippocampus and make inferences for patients with irritable bowel syndrome. The involvement of the HPA-axis was implicated by the change in serum corticosterone level.

Using the psychological stress of maternal separation, Gareau *et al.* (2007) showed that Sprague-Dawley rat pups separated from the dam for three hours per day had increased serum corticosterone and increased intestinal permeability when compared to sham controls. Administering Lacidofil to the pups normalised all the serum corticosterone and the gut permeability. The authors state that the amelioration of the gut dysfunction is mediated through the HPA-axis.

In another model of psychological stress, Zareie *et al.* (2006) applied water avoidance stress (WAS) to adult male Brown Norway rats to determine if Lacidofil could prevent WAS-induced intestinal pathophysiology. The probiotic prevented stress-induced bacterial adherence to rat enterocytes in both the ileum and colon, and eliminated bacterial translocation to the mesenteric lymph nodes. Interestingly, the lactobacilli administration inhibited the elevated intestinal ion secretion but not the increased permeability. The possible impact on visceral hypersensitivity was discussed by the authors.

Overall, these studies demonstrate that the probiotic Lacidofil reduced stress-induced responses, such as increased gut permeability, inflammatory and serum corticosterone levels. The impact of the probiotic seems to be mediated through the HPA-axis.

8. Studies in humans

Firmesse *et al.* (2008) investigated the ability of R0011 and R0052 strains to survive passage through the human digestive tract. Healthy study participants were given four capsules of Lacidofil (each containing 1.9×10^9 R0011 and 1×10^8 R0052) daily for 12 days, followed by a 12-day washout period. Quantification of bacterial strains in faecal samples demonstrated that R0011 survived transit and was present in faeces at high concentrations (7.1×10^{10} cfu/g of stool), but it was rapidly eliminated after the end of the consumption period with no lasting colonisation. R0052 was not detected at significant levels in the faecal samples, suggesting it either did not survive or, given the highly adherent nature of the bacteria, it was associated with the mucosal layer and not the luminal microbiome. The R0052 may be gradually eliminated with time but at levels below the detectable threshold. Intestinal biopsy sampling would prove useful for clarifying the location of this bacterium in future studies. No changes were seen in the overall microbiota profile of participants which was not surprising given the study group was composed of healthy individuals with presumably a balanced gut microbiome.

9. Clinical studies of gastrointestinal diseases

The majority of clinical studies investigating Lacidofil examine its use in improving gastrointestinal health in patients with dysbiosis. Table 4 summarises the clinical studies of antibiotic-associated diarrhoea (AAD), *H. pylori* eradication, and paediatric gastrointestinal disorders. Antibiotic therapy may be a cause of dysbiosis due to the suppression of commensal microorganisms and the excessive growth of pathogens such as *Clostridium difficile.* Provision of *Lactobacillus* spp. in combination with antibiotics may prevent C. *difficile* or other pathogenic infection by restoring intestinal microbiome (D'Souza *et al.,* 2002). Maydannik *et al.* (2010) conducted a randomised, controlled study of 214 children being treated with antibiotics for respiratory, urinary, or digestive illnesses. The children receiving Lacidofil (one to three capsules/day, 2×10^9 cfu/capsule) experienced a 1.5-fold lower incidence of AAD, and a two-fold decrease in duration of diarrhoea, 8-fold decrease in *C. difficile* toxins, and a 10-fold decrease in *C. difficile* carriage compared to children on antibiotics alone. Lacidofil treatment resulted in an 84.5% decrease of *C. difficile* toxins in children and no reported side effects. A randomised, controlled study of 59 children with pulmonary tuberculosis by Patsera *et al.* (2010) demonstrated that Lacidofil reduced *C. difficile* carriage. Patients treated with conventional therapy and the probiotic at a dose of 6×10^9 cfu/day for one month experienced a significant increase

Table 4. Clinical studies of antibiotic-associated diarrhoea, *Helicobacter pylori* eradication and paediatric gastrointestinal disorders.

Reference	Study type	Study size[2]	Dosing regime[1]	Results[2]
Antibiotic-associated diarrhoea				
Aryayev and Kononenko, 2009	randomised controlled	36 children with mucoviscidosis (18 conventional therapy with Lacidofil + 18 conventional therapy alone)	age <12 months: 1 capsule qd, 1-3 yrs: 1 capsule bid, 3-12 yrs: 1 capsule tid, >12 yrs: 2 capsules tid	decreased AAD development in children with Lacidofil; improved intestinal microbial balance
Marushko *et al.,* 2007	randomised controlled	34 children aged 10 months-3 yrs with respiratory pathology (16 antibacterial therapy with Lacidofil + 16 antibacterial therapy alone)	age <1 yr: 1 capsule qd, 1-3 yrs: 1 capsule bid for 2-4 weeks	decreased incidence and duration of AAD; more normalised intestinal microbiome following therapy
Maydannik *et al.,* 2010	randomised controlled	244 children aged 0-17 yrs with acute respiratory, urinary, or digestive exacerbations (117 antibiotic therapy with Lacidofil + 127 antibiotic therapy alone)	age <1 yr: 1 capsule qd, 1-3 yrs: 1 capsule bid, 3-12 yrs: 1 capsule bid or tid, >12 yrs: 1-2 capsules tid for 14-21 days	lower incidence/decreased duration of AAD with Lacidofil; decreased *Clostridium difficile*/toxin carriage with Lacidofil
Patsera *et al.,* 2010	randomised controlled	59 children aged 3-18 yrs with pulmonary TB (23 anti-TB chemotherapy with Lacidofil + 36 anti-TB chemotherapy alone)	3 capsules/day for 1 month; 6 capsules/day for 1 month	reduced C. *difficile* toxins in stool with 6 capsules/day; alleviated gastrointestinal symptoms; normalised stool; disappearance of glossitis
Song *et al.,* 2010	randomised controlled double-blind	214 adults with respiratory tract infections on antibiotics (103 Lacidofil + 111 placebo)	1 capsule bid for 14 days vs. placebo (1 capsule bid for 14 days)	improved bowel frequency and consistency with Lacidofil; no difference in AAD occurrence (possibly too small dose of Lacidofil given)
Helicobacter pylori eradication				
Babak, 2007	randomised controlled	35 adults with *H. pylori* associated duodenal ulcer; (20 RAB/AMO/CLA with Lacidofil + 15 RAB/AMO/CLA alone)	2 capsules tid for 20 days	increased eradication of *H. pylori* with Lacidofil; faster alleviation of dyspeptic symptoms; improved duodenal mucous coat; improved restoration of intestinal microbiota
Gnaytenko *et al.,* 2009	non-randomised controlled	45 children with *H. pylori* (25 anti-*Helicobacter* therapy with Lacidofil + 20 anti-*Helicobacter* therapy alone)	1 capsule bid for 20 days	decreased incidence of AAD with Lacidofil
Vdovychenko *et al.,* 2008	controlled (randomised?)	49 adults with *H. pylori* (25 triple therapy with Lacidofil + 24 triple therapy alone)	2 capsules bid for 10 days	increased eradication of *H. pylori; i*ncreased healing of ulcers
Ziemniak, 2006	open label	641 adults with *H. pylor*i (192 PPI/CLA/AMO + 241 PPI/TET/TNZ/bismuth salts + 53 according to antibiogram + 102 according to antibiogram + 53 PPI/CLA/AMO with Lacidofil)	2 capsules bid for 20 days	increased eradication of *H. pylori* with Lacidofil
Paediatric gastrointestinal diseases				
Skorodumova *et al.,* 2007	controlled, matched for age, sex, duration of disease, aetiology and severity of acute intestinal infections	248 paediatric patients with acute intestinal infection (225 conventional treatment + 23 conventional treatment with Lacidofil)	1 capsule tid for 14 days	restored intestinal microbiome with probiotic; improved immune status (increased phagocytosis); increased IgA/IgG) with probiotic

Table 4. Continued.

Reference	Study type	Study size[2]	Dosing regime[1]	Results[2]
Paediatric gastrointestinal diseases (continued)				
Tlaskal *et al.*, 1995	nonrandomised, controlled	75 paediatric patients (33 treated with R0052/R0011 + 42 control)	dosage varied by patient for 14 days	faster recovery from AAD with probiotic; decreased incidence of opportunistic *Citrobacter freundii*
Tlaskal *et al.*, 2005	randomised controlled, double blind	113 paediatric patients (39 placebo + 42 receiving Lacidofil + 32 receiving Hylac-concentration of metabolic products of common intestinal symbiotic bacteria)	1 capsule qd for 10 days	decreased duration of stool consistency with Lacidofil; no change was seen in total IgA in the saliva or stools for either treatment group

[1] qd = *quaque die* or once per day; bid = *bis in diem* or twice daily; tid = *ter in diem* or three times daily.
[2] AAD = antibiotic-associated diarrhoea; AMO = amoxicillin; CLA = clarithromycin; PPI = proton pump inhibitor; RAB = rabeprazole; TB = tuberculosis; TET = tetracycline; TNZ = tinidazole.

in the amount of *C. difficile* toxins A + B in the stool (from 1.1±0.2 ng/ml to 2.4±0.4 ng/ml, $P<0.05$), whereas patients treated with conventional therapy and probiotics at a dose of 12×10^9 cfu/day for one month experienced a significant reduction of toxins A + B in stool (from 1.53±0.3 ng/ml to 0.93±0.1 ng /ml, $P<0.01$). Additionally, patients on Lacidofil experienced improved appetite, normalised stool, reduced gastrointestinal discomfort, and the disappearance of glossitis during the course of treatment.

A controlled study by Marushko *et al.* (2007) found Lacidofil reduced the side effects of antibiotic treatment in 34 children. Antibiotic treatments included in the study were semi-synthetic penicillins (amoxicillin, amoxiclav), cephalosporins of the 1st and 2nd generation (cefazolin, cefuroxime), and macrolides (sumamed). AAD occurred in 2 of 16 participants (12.6%) receiving Lacidofil, compared to 8 of 18 (44.8%) in the control group ($P<0.05$). In addition, the duration of diarrhoea was significantly shorter in the treatment group (2.6±1.1 days) compared to the control group (5.9±1.2 days) ($P<0.05$).

In a study by Song *et al.* (2010), 214 adult patients being treated with antibiotics for respiratory tract infections were randomised to receive Lacidofil or a placebo for 14 days. The number of adults who developed AAD in this study was low. AAD developed in 4 (3.9%) of 103 patients in the Lacidofil group and in 8 (7.2%) of 111 patients in the placebo group ($P=0.44$). Although this was not considered a significant difference between groups, the Lacidofil group did experience less change in bowel frequency and consistency (50/103, 48.5%) than the placebo group (35/111, 31.5%) ($P=0.01$).

In a controlled study of 36 children with upper respiratory infection, Aryayev and Konenko (2009) found an 80% risk reduction of AAD when Lacidofil was added to antibiotic treatment. AAD development was 5.6% in the group receiving Lacidofil, and 28% in the control group.

Patients being treated with antibiotics for *H. pylori* infections may also benefit from Lacidofil. Gnaytenko *et al.* (2009) investigated the effects of Lacidofil in 45 children receiving anti-helicobacter therapy. Children given Lacidofil in combination with amoxicillin and clarithromycin had a significantly lower incidence of AAD (8.0±5.5%) compared to patients treated without probiotics (35.0±10.9%) ($P<0.05$). Additionally, *C. difficile* toxins were found in the stool of five children (25%) treated without probiotics, but in only one (4.0%) child given Lacidofil ($P<0.05$). Thus, administration of Lacidofil from the first day of anti-helicobacter therapy reduced symptoms and prevented colonisation of the digestive tract by toxin-forming *C. difficile*.

A randomised, controlled study of 641 adult patients with *H. pylori* infections by Ziemniak (2006) revealed that when Lacidofil was added to conventional triple therapy, it not only improved symptoms of the conventional therapy, but also increased eradication of *H. pylori* from 85.9% to 94.3% ($P<0.05$). Similar results were found in a smaller, controlled study of 49 adults with *H. pylori* infection by Vdovychenko *et al.* (2008), 96.0% eradication with Lacidofil compared to 75.0% control. Moreover, an increase in ulcer healing was seen with Lacidofil, 88.0% healing with Lacidofil compared to 70.8% in the conventional therapy group. Results from a controlled study of 35 adult patients with *H. pylori* associated duodenal ulcers (Babak, 2007) also showed a reduction in *H. pylori* when Lacidofil was added to conventional treatment. Eradication was seen in 18 of 20 (90±6.7%) patients receiving Lacidofil and in 13 of 15 (86.7±8.8%) patients in the control group. Furthermore, dyspeptic symptoms were corrected faster in the treatment group (6.0±0.6 days) compared to the control group (10.0±1.1 days) ($P<0.01$).

Due to the high incidence of diarrhoea and intestinal infections in infants and children, many researchers have investigated the use of Lacidofil in the paediatric population. Tlaskal *et al.* (1995) found that children with gastrointestinal diseases treated orally with the probiotic experienced significantly faster recovery (4.8±4.9 days) compared to children treated without the probiotic (8.7±4.2 days) (P<0.01). Recovery was determined by complete decline of clinical symptoms (number of stools, stool consistency, vomiting, stomach pain, fever, lack of appetite) and a negative test for the pathologic agent if originally present. Furthermore, an improvement in the intestinal microbiome was seen. Specifically, there was a reduced carriage of the opportunistic pathogen, *Citrobacter freundii*. This microbe has been associated with non-gastrointestinal nosocomial infections of the respiratory tract, urinary tract, blood and other organs (Lockhart *et al.*, 2007). Other studies have examined the effects of Lacidofil on the immune status in infants and children. A controlled study of 248 paediatric patients with acute intestinal infection demonstrated that Lacidofil supplementation (1 capsule, 3 times daily for 14 days) increased phagocytosis, as well as levels of IgA (0.59±0.12 g/l before treatment, 0.68±0.08 g/l after treatment) and IgG (6.34±0.31 g/l prior to treatment, 7.62±0.43 g/l post treatment) (Skorodumova *et al.,* 2007). Conversely, a controlled study by Tlaskal *et al.* (2005) showed no significant increase in saliva or stool IgA concentrations after a lower dose of Lacidofil supplementation (1 capsule, once daily for 10 days) in infants with acute gastroenteritis. The authors did, however, find a reduction in the duration of diarrhoea from 5.45±2.33 days to 4.00±2.02 days with Lacidofil (P<0.05). Further research is needed to investigate the conflicting results regarding IgA.

Several studies have shown a positive outcome in irritable bowel syndrome (IBS) with probiotic supplementation. Clinical studies of Lacidofil on IBS are summarised in Table 5. While it is not well established, some researchers have suggested that IBS is associated with a significant decrease in faecal *Bifidobacterium* (Malinen *et al.,* 2006) and an increase in faecal *Enterobacteriaceae* (Si *et al.,* 2004). However, it must be clear that the patterns may significantly vary among different phenotypes of this heterogeneous functional disorder (diarrhoea, constipation, bloating, pain, etc.). In an open label study conducted by Zvyagintzeva and Plutenko (2008), researchers found that Lacidofil supplementation (1 to 3 capsules/day for 3 weeks) restored eubiosis in 85% of patients and significantly improved the microbiotic composition in the remaining 15% of patients with dysbiosis related to IBS. However, the relevance of these observed changes is not fully understood in this study group. After the treatment course, dyspeptic symptoms (abdominal pain, creatorrhoea, steatorrhoea, amylorrhoea, and polyfaecalia) disappeared in 18 of 20 patients (90%), although meteorism was occasionally observed in three patients (15%).

In an open label study by Benes *et al.* (2006), improved clinical symptoms were observed when Lacidofil was given to IBS patients with long standing symptoms. Fifty patients with chronic IBS were given 1 capsule 3 times daily

Table 5. Clinical studies of irritable bowel syndrome and lactose intolerance.

Reference	Study type	Study size[2]	Dosing regime[1]	Results[2]
Irritable bowel syndrome				
Benes *et al.,* 2006	open label, uncontrolled	50 adults with IBS	1 capsule tid for 4 months	decreased frequency of defecation; improved stool consistency; reduced abdominal pressure and bloating; decreased flatulence
Zvyagintzeva, 2008	open label	20 adults with IBS and medium severity diarrhoea	1 capsule tid for 14 days followed by 1 capsule qd for 7 days	improved composition of intestinal microorganisms; decreased number of *Candida; i*mproved clinical symptoms
Lactose intolerance				
Kocian, 1994	open label	21 adults with lactose intolerance	1 capsule qd for 2 weeks	improved lactose tolerance; improved consistency of stool; decreased frequency of defecation
Rampengan *et al.,* 2010	randomised controlled, single-blind	79 children aged 10-12 yrs with lactose intolerance (39 live probiotic/ Lacidofil + 40 killed probiotic/Dialac)	1 capsule qd for 14 days vs. Dialac (2 sachets qd for 14 days)	significantly reduced BHT with both live and killed probiotics; improved lactose tolerance with both live and killed probiotics

[1] qd = *quaque die* or once per day; tid = *ter in diem* or three times daily.
[2] IBS = irritable bowel syndrome; BHT = breath hydrogen test.

for 4 months, and re-examined four months after ending treatment. Frequency and consistency of stools improved in 42 patients (84%), and flatulence was reduced in 31 patients (62%). Twenty patients experienced a reappearance of symptoms within four months of finishing treatment, of whom 15 patients requested to resume the probiotic supplementation. While these studies have suggested possible benefits of Lacidofil in the treatment of IBS, large-scale, randomised controlled studies are required to verify the results.

A possible action of lactobacilli in the intestinal tract is to enhance the digestion of lactose (Rampengan *et al.*, 2010). Based on this function, Lacidofil has been considered to alleviate symptoms of lactose maldigestion. The clinical studies to date are presented in Table 5. Kocian (1994) found that patients with lactose intolerance experienced improvement in symptoms after treatment with Lacidofil (under its old trade name in the Czech Republic, '*L. acidophilus*'). Stool frequency was reduced by 0.57 bowel movements per day ($P<0.01$), and consistency improved an average of 1.37 points on a scale of 0 to 3 ($P<0.001$). Participants were also able to tolerate more lactose-containing foods following the lactobacilli supplementation.

A randomised, controlled study by Rampengan *et al.* (2010) compared the use of live (Lacidofil capsule) and heat-killed (Dialac® sachet) probiotics in treating symptoms of lactose maldigestion. Administration of both live and killed probiotic showed no difference in breath hydrogen test results between the two treatments (22.13±12.41 mg/kg after Lacidofil; 20.30±8.86 mg/kg after Dialac; $P=0.453$). However, symptoms of lactose maldigestion improved with both treatments. Researchers concluded that there was no significant difference in the efficacy of live versus dead strains in managing symptoms of lactose maldigestion.

10. Clinical studies of atopic dermatitis

Three clinical trials, presented in Table 6, have been carried out on Lacidofil and atopic dermatitis (AD). AD is a skin disease characterised by an abnormal immune response often correlated with a food allergy (milk or egg). The cause of AD is not fully understood, but activated T- and B-cells have been identified as a significant contributor to pathogenesis (Chernyshov, 2009a). B7-1 (CD80) and B7-2 (CD86), members of the immunoglobulin superfamily, are responsible for the activation of T-cells by binding to the receptors CD28 and CTLA4. Hence, they have a dominant role in co-stimulatory pathways that regulate T- and B-cell response. Because of this prominence, they have complementary roles in mediating allergic pulmonary inflammation (Mark *et al.*, 2000). Current treatment of atopic dermatitis involves conventional anti-inflammatory preparations, but research in mouse models has indicated that probiotics may be beneficial by modulating immune responses through the suppression of B7-2 (Inoue *et al.*, 2007). It is constitutively expressed in a number of dendritic cells, B-cells, Langerhans cells and is expressed at low levels in monocytes. It can be upregulated through interferon gamma.

Chernyshov (2009a) investigated the B7-2/CD28 interaction in AD and correlations with total and specific IgE, interleukin 4 (IL-4), and interferon-gamma (IFN-γ) production during a one month follow up. Paediatric patients were given Trixera® emollient cream and Trixera emollient preparation for bath as moisturisers in combination with Lacidofil (1 capsule daily). Results of the study supported the theory of CD28/B7 stimulation in children with AD. CD28 was correlated to the IL-4 and IFN-γ cytokines, but no relationship was found between B7-2 expression and serum IgE (a causative factor in AD exacerbation). The observation of non-correlation with B7-2 is not unexpected given that it is constitutively expressed in most cells.

Additional studies have been conducted by Chernyshov to investigate the effects of probiotics on the clinical symptoms of AD (represented by SCORAD – SCORing Atopic Dermatitis – clinical tool) and the quality of life for patients and their families. In one report, 36 children up to age 4 with AD were given Trixera emollient with Lacidofil (1 capsule daily) (Chernyshov, 2007). After one month of treatment, SCORAD improved from 40.82±4.00 to 24.67±3.32 ($P<0.001$). Additionally, quality of life ratings were significantly greater following treatment ($P<0.05$). In a separate study, 58 children with AD up to age 4 were randomised to receive either Trixera emollient and Lacidofil (main group) or Trixera emolient alone (control group) (Chernyshov, 2009b). Children treated with Lacidofil experienced a greater reduction in clinical symptoms than children treated with Trixera alone. SCORAD was reduced by an average of 63.3% in the treatment group and only 32.1% in the placebo group ($P=0.02$). Additionally, Lacidofil increased IgG4 to milk proteins (128.35±21.56 IU/ml before treatment; 141.57±23.09 IU/ml after treatment, $P<0.001$), signifying improved tolerance, and decreased activation of T-cells (42.77±4.42% before treatment; 45.83±4.72% after treatment, $P<0.05$), indicating a reduced immune response.

Research on this topic is limited, and more investigation should be done to fully understand the relationship between lactobacilli and immune system modulation in AD.

11. Clinical studies of vaginal dysbacteriosis

With caesarean section delivery, prophylactic antibiotic therapy is used and may influence the development of maternal dysbacteriosis. Two trials investigating the impact of Lacidofil on vaginal dysbacteriosis have been carried out (Table 6). In a randomised, controlled study of 96 women

Table 6. Clinical studies of non-gastrointestinal disorders.

Reference	Study type	Study size[2]	Dosing regime[1]	Results
Atopic dermatitis				
Chernyshov., 2007	open label	36 children with AD up to age 4 (Trixera + Lacidofil)	1 capsule qd	improved SCORAD; improved quality of life in patients and parents
Chernyshov, 2009a	open label	24 children up to age 4 with AD and cow's milk allergy (Trixera emollient w/ Lacidofil)	1 capsule qd	significant correlation between CD28 and B7-2 on CD19+ B-cells; CD28 expression was well correlated with clinical severity of AD; significant correlation between CD28 and both IL-4 and IFN-γ before treatment only; increased IgG4 levels and decreased SCORAD after treatment; poor correlation between B7 and both IgE and IL-4 production
Chernyshov, 2009b	randomised, controlled	58 children with AD up to age 4 (30 Lacidofil with Trixera + 28 Trixera alone)	1 capsule qd	improved SCORAD with Lacidofil; increased IgG4 to cow milk with Lacidofil
Vaginal dysbacteriosis				
Chayka *et al.,* 2006	controlled, matched for social status, education level, parity, general and gynaecological anamnesis	103 pregnant women (38 pre- and postoperative Lacidofil + 35 postoperative Lacidofil + 30 control)	1 capsule bid 5-6 days preoperative and 10 days postoperative	reduced opportunistic microbiome (esp. *Candida*) with preventative Lacidofil; decreased colonisation of amniotic fluid and GI tract of newborn with Lacidofil
Liskovich *et al.,* 2010	controlled, matched for age, anthropometric data, frequency of gynecological and extragenital pathology	96 women post-caesarean operation (56 cefotaxime with Lacidofil-WM + 40 cefotaxime alone)	1 capsule tid for 7 days	decreased incidence of vaginal dysbiosis with Lacidofil; prevention of AAD with Lacidofil

[1] qd = *quaque die* or once per day; bid = *bis in diem* or twice daily; tid = *ter in diem* or three times daily.
[2] AD = atopic dermatitis; GI = gastro-intestinal tract.

receiving prophylactic antibiotic therapy after caesarean section delivery (Liskovich *et al.,* 2010), 89.3% of patients receiving Lacidofil supplementation were considered eubiotic (i.e. having a balanced microbiome) following therapy, while none of the patients of the control group were eubiotic. Antibiotic-associated diarrhoea did not develop in any of the patients receiving Lacidofil, but AAD was recorded in 10% of patients from the control group.

Chayka *et al.* (2006) compared the use of Lacidofil administered following caesarean section delivery to Lacidofil administered 7 to 10 days prior to and following delivery. The results of this study demonstrated that Lacidofil supplementation as part of a preventative treatment before a caesarean delivery may reduce the incidence of opportunistic microbiome up to 3-fold, while Lacidofil given postoperatively only reduced incidence 1.3-fold. Both treatment regimens decreased development of dysbiosis in the mother and infant.

12. Adverse events monitoring during clinical evaluation

The discussion of potential side effects of Lacidofil in the reviewed studies was not well-developed. Five studies reported no undesired outcomes when monitoring for side effects such as allergic reactions or intolerance (Aryaev and Konenko, 2009; Benes *et al.,* 2006; Marushko *et al.*, 2007; Maydannik *et al.*, 2010, Rampengan *et al.,* 2010). Song *et al.* (2010) noted mild abdominal pain in three patients and a skin eruption in one patient receiving Lacidofil, but concluded that these adverse events could not be attributed to the use of Lacidofil. Caution should be taken when administering live probiotics to severely ill or immune-compromised patients as they may be more susceptible to unfavourable outcomes (Besselink *et al.,* 2008). More research is needed to investigate the full impact of long-term supplementation with Lacidofil, although no harmful consequences have been recorded to date.

13. Comparison of outcomes with Lacidofil® to systematic meta-analyses of probiotics

No systematic reviews have focused specifically on the outcomes associated with the R0052 and R0011 strains present in Lacidofil. However, a meta-analysis by Cremonini *et al.* (2002) evaluated probiotic preparations of *S. boulardii* or *Lactobacillus* species as a therapy for AAD. While there was significantly reduced incidence of diarrhoea with probiotic administration, (relative risk 0.40, 95% confidence interval (CI) = 0.28-0.57), the reviewers acknowledged that different strains possess different characteristics, and more clinical studies are needed to identify the properties of specific probiotic strains. Four randomised, controlled studies included in the analysis used *Lactobacillus* GG preparations, and each produced similar results to those found by randomised, controlled studies of Lacidofil included in our review. For example, Arvola *et al.* (1999) found *Lactobacillus* GG supplementation reduced AAD occurrence from 16% in the control group to only 5%, while Aryayev and Konenko (2009) found Lacidofil supplementation reduced AAD occurrence from 28% to 5.6%.

A Cochrane review by Allen *et al.* (2008) evaluated the use of probiotics in the treatment of infectious diarrhoea. All included studies tested various lactobacilli preparations, except two studies which tested the yeast *S. boulardii.* The reviewers determined that probiotics reduced the risk of diarrhoea at 3 days (relative risk 0.66, 95% confidence interval 0.55 to 0.77, random effects model; 15 studies) and the average duration of diarrhoea by 30.48 hours (95% confidence interval 18.51 to 42.46 hours, random effects model, 12 studies). A 2002 publication by Van Niel *et al.* (2002) reviewed nine clinical studies of *Lactobacillus* on the treatment of infectious diarrhoea in the paediatric population. They concluded that *Lactobacillus* therapy is a safe and cost-effective way to reduce the duration of diarrhoea (0.7 days, 95% confidence interval: 0.3-1.2 days) and frequency of defaecation (1.6 stools on day 2 of treatment, 95% confidence interval: 0.7-2.6 fewer stools) in children. These results are similar to the values found with Lacidofil supplementation. Tlaskal *et al.* (2005) found a reduction in the duration of diarrhoea by an average of 1.45 days in children with diarrhoeal disease.

Tong *et al.* (2007) reviewed 14 clinical trials to evaluate the therapeutic value of various probiotic preparations on *H. pylori* infections. Authors concluded that in combination with conventional treatment, probiotics were effective in increasing eradication of *H. pylori* (odds ratio 1.82; 95% CI=1.30-2.56). Eradication rates were 83.6% (95% CI=80.5-86.7%) for patients treated with probiotics and 74.8% (95% CI=80.5-86.7%) for patients treated without probiotics. Improvements in *H. pylori* eradication with Lacidofil were similar, increasing from 85.9% to 94.3% (Ziemniak, 2006), from 75.0% to 96.0% (Vdovychenko *et al.*, 2008), and from 86.7% to 90.0% (Babak, 2007).

McFarland and Dublin (2008) reviewed 20 clinical trials with 23 different probiotic preparations for the treatment of IBS (including *L. rhamnosus* and *L. acidophilus* preparations). They determined that current research shows potential therapeutic benefits for IBS, with overall reduction of global symptoms (relative risk 0.77, 95% CI=0.62-0.94), but they also emphasised that more research is needed before recommendations for clinical practice can be made. Again, this reiterates the results found from the reviewed studies on Lacidofil. Despite the observed positive outcomes with Lacidofil supplementation, there is a lack of large, controlled studies on this topic.

The FAO and WHO expert consultation committee state that the beneficial effects observed with one strain cannot be assumed to occur with other strains (FAO/WHO, 2001). It is therefore ideal to assess the health consequences of probiotic strains or products individually. Lacidofil has demonstrated similar results to meta-analyses outcomes of various probiotic strains and products. Of the 20 clinical studies reviewed, the most convincing evidence for benefits of Lacidofil were seen for treatment of AAD, *H. pylori* infection, and paediatric gastrointestinal diseases. Positive outcomes were also seen for IBS and lactose intolerance, however, the evidence was not as strong, and research on these topics is limited. Additionally, the use of Lacidofil to treat atopic dermatitis and vaginal dysbacteriosis has shown potential benefits. However, both of these topics require further investigations before conclusions can be made for clinical recommendations.

14. Conclusions

The strains in Lacidofil appear to interact and influence the host through distinct but mutually beneficial mechanisms. Mechanistic and animal data suggest they directly influence pathogen-host interaction, modulate the immune pathways primarily by down-regulating pro-inflammatory responses and they help maintain the protective gut barrier. Research collected from clinical studies of Lacidofil indicate that it may have a beneficial impact on the intestinal, vaginal, and skin microbiomes when ingested in sufficient quantities. Supplementation can alleviate symptoms of diarrhoea caused by antibiotic therapy, irritable bowel syndrome, or lactose intolerance. Given as a co-therapy for atopic dermatitis, lactobacilli have demonstrated positive effects on immune responses and milk tolerance in patients.

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