World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v20.i3.724

World J Gastroenterol 2014 January 21; 20(3): 724-737
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (6): *Helicobacter pylori*

Efficacy of fermented milk and whey proteins in *Helicobacter pylori* eradication: A review

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Author contributions: Nagpal J conceived the idea of the manuscript; Sachdeva A and Rawat S conducted the literature search, rated the studies and drafted the manuscript; Nagpal J finalized the manuscript and will act as guarantor for the paper.
Supported by Intramural funding by Sitaram Bhartia Institute of Science and Research, New Delhi
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Received: June 29, 2013 Revised: November 9, 2013
Accepted: December 5, 2013
Published online: January 21, 2014

Abstract

Helicobacter pylori (*H. pylori*) eradication is considered a necessary step in the management of peptic ulcer disease, chronic gastritis, gastric adenocarcinoma and mucosa associated lymphoid tissue lymphoma. Standard triple therapy eradication regimens are inconvenient and achieve unpredictable and often poor results. Eradication rates are decreasing over time with increase in antibiotic resistance. Fermented milk and several of its component whey proteins have emerged as candidates for complementary therapy. In this context the current review seeks to summarize the current evidence available on their role in *H. pylori* eradication. Pertinent narrative/systematic reviews, clinical trials and laboratory studies on individual components including fermented milk, yogurt, whey proteins, lactoferrin, α-lactalbumin (α-LA), glycomacropeptide and immunoglobulin were comprehensively searched and retrieved from Medline, Embase, Scopus, Cochrane Controlled Trials Register and abstracts/proceedings of conferences up to May 2013. A preponderance of the evidence available on fermented milk-based probiotic preparations and bovine lactoferrin suggests a beneficial effect in Helicobacter eradication. Evidence for α-LA and immunoglobulins is promising while that for glycomacropeptide is preliminary and requires substantiation. The magnitude of the potential benefit documented so far is small and the precise clinical settings are ill defined. This restricts the potential use of this group as a complementary therapy in a nutraceutical setting hinging on better patient acceptability/compliance. Further work is necessary to identify the optimal substrate, fermentation process, dose and the ideal clinical setting (prevention/treatment, first line therapy/recurrence, symptomatic/asymptomatic, gastritis/ulcer diseases *etc.*). The potential of this group in high antibiotic resistance or treatment failure settings presents interesting possibilities and deserves further exploration.

Key words: *Helicobacter pylori*; Fermented milk; Whey proteins; Bovine lactoferrin; α-Lactalbumin; Glycomacropeptide; Immunoglobulin

Core tip: Treatment regimens for *Helicobacter* are cumbersome, prone to side effects and often have low success rates. Fermented milk and related proteins have often been explored as potential candidates for complementary therapy. The current review sought to summarize the current evidence available on their role in *Helicobacter pylori* eradication and found substantial evidence to support the use of fermented milk based probiotic preparation and bovine lactoferrin. Evidence for other whey proteins is preliminary and requires substantiation. Further work is necessary to identify the optimal substrate, fermentation process, dose and

the ideal clinical setting. The potential of this group in antibiotic resistance or treatment failure settings also presents interesting possibilities.

Sachdeva A, Rawat S, Nagpal J. Efficacy of fermented milk and whey proteins in *Helicobacter pylori* eradication: A review. *World J Gastroenterol* 2014; 20(3): 724-737 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i3/724.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i3.724

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram negative, spiral shaped bacterium found in the gastric mucous layer. It has an ammonia-producing surface urease which allows adherence to and colonization of the gastric epithelium, by neutralizing the acidic gastric environment[1]. *H. pylori* is now implicated in peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, mucosa associated lymphoid tissue lymphoma and duodenal ulcer disease[2-4]. Eradication of *H. pylori* is considered a necessary step in the management of these diseases. Standard triple therapy eradication regimens (proton pump inhibitor plus clarithromycin and amoxicillin or nitroimidazole) are inconvenient and achieve unpredictable and often poor results[5]. Further, eradication rates are reported to be decreasing over time with an increase in antibiotic resistance[6]. Second line quadruple regimens are further limited by poorer patient compliance and increased side effects[6]. In this context, several alternative and complementary therapies have been tried in an attempt to achieve better eradication without affecting compliance. In this search, fermented milk and several of its component whey proteins have emerged as potential candidates for complementary therapy. They have the inherent advantage of better patient acceptability.

Several randomized controlled trials and a recent meta-analysis document that fermented milk-based probiotic preparations improve *H. pylori* eradication rates by 10%. Their efficacy has been argued to be better than capsule-based bacteria-only preparations and considered partly or completely contributed by the anti-bacterial and immunogenic properties of component whey proteins formed as a result of fermentation *etc*. Potential efficacy of individual whey proteins in *H. pylori* eradication has also been a subject of interest in recent research. However the role of fermented milk or whey proteins in clinical practice is not yet universally accepted, precisely defined or widely discussed[7]. In this context the current review sought to summarize the current evidence available on the role of fermented milk and its component whey proteins in *H. pylori* eradication.

For the purpose of the current review pertinent narrative/systematic reviews, clinical trials and laboratory studies on individual components including fermented milk, yogurt, whey proteins, lactoferrin, α-lactalbumin (α-LA), glycomacropeptide and immunoglobulin were comprehensively searched and retrieved from Medline, Embase, Scopus, Cochrane Controlled Trials Register and abstracts/proceedings of conferences up to May 2013. The available studies/meta-analysis were rated for quality as per the Scottish Intercollegiate Guidelines Network (SIGN) check lists[8] and the Quality Rating for Individual Studies[9]. The evidence was subsequently graded using the Revised Grading System[10]. The level of recommendation was later defined into one of four grades (A, B, C or D; SIGN grades)[11].

FERMENTED MILK

Fermented milk refers to whole or skimmed milk curdled to a beverage or custard like consistency by lactic acid producing bacteria. A wide assortment of products, varying by the process, bacteria, duration and other variables, are available and widely consumed in different countries. However, there are several commonalities. Fermented milk possesses a protein system constituted by two major families of proteins *i.e.*, casein and whey proteins. Casein is insoluble, and accounts for 80% of the whole protein inventory. Whey proteins are globular water soluble molecules and include bovine lactoferrin, α-LA, glycomacropeptide, immunoglobulin, β-lactoglobulin and lactoperoxidase. Whey is thought to have the ability to act as an antioxidant, immune enhancer, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial and as a chelating agent[12].

In the context of Helicobacter eradication there is a fair body of evidence from trials conducted using fermented milk (usual culturally/commercially available preparations including yogurt), fermented milk based probiotic preparations (FMPPs; fermented milk with specifically added live probiotic bacteria like Lactobacilli) and capsule based probiotics. An observational study on 464 healthy Mexican subjects documented lower prevalence of *H. pylori* seropositivity in those consuming yogurt more than once a week compared with non-consumers[13]. As presented in Table 1, several clinical trials and a systematic review of RCTs compared an FMPP *vs* placebo or standard therapy plus FMPP *vs* standard therapy and documented a beneficial effect of FMPPs[14]. The overall quality and quantity of evidence for FMPPs appears convincing (Recommendation Grade-A) and beneficial effect appears to be sustained when FMPP were used in combination with standard therapy (Recommendation Grade A[15-18]). Also, benefit has been documented in symptomatic children (Recommendation Grade-B), symptomatic and asymptomatic adults (Recommendation Grade-B) and in patients who failed eradication on standard therapy (Recommendation Grade-B). The overall magnitude of the benefit was estimated to be 5%-15%[14].

With reference to the active principle components responsible for this effect, the available clinical evidence can be better summarized on the basis of three argu-

Table 1 Studies comparing "fermented milk based probiotic preparation" with placebo or "standard therapy + fermented milk based probiotic preparation" with "standard therapy"

Ref.	Type of trial	Evidence grade[1]	Quality rating[2]	Subjects	Study design	Study groups/methods	Outcome variable/s	Results and conclusions
Positive								
Bekar *et al*[15], 2011, Turkey	Human	1+	+	82 pts of dyspepsia and *H. pylori* infection	RCT	Two groups - Control group (n = 36; Triple therapy - lansoprazole, clarithromycin and amoxicillin + placebo) and Treatment group [n = 46; Triple therapy + kefir (fermented milk drink containing probiotics)]; given for 14 d	Eradication of *H. pylori*; adverse events of eradication therapy (Urease test after 45 d of treatment)	Significantly more patients (78.2% *vs* 50.0%) in the treatment group achieved eradication in comparison with control group. Side effects were less frequent and less severe in the treatment group
Sachdeva *et al*[14] 2009, India	Metaanalysis	1+	++	10 eligible trials; data available for 963 patients	Meta-analysis of human RCTs/ CCTs	Trials had to be randomized or quasi-randomized and controlled, using a FMPP in the intervention group treating *Helicobacter*-infected patients. The only difference between the two groups had to be FMPP	Eradication of *H. pylori*; adverse events of eradication therapy	The pooled odds ratio for eradication by ITT analysis in the treatment *vs* control group was 1.91 (1.38-2.67; P < 0.0001) using fixed effect model The pooled risk difference was 0.10 (95%CI: 0.05-0.15; P < 0.0001) by fixed effect model. Fermented milk based probiotic preparations improve *H. pylori* eradication rates by approximately 5%-15%, whereas the effect on adverse effects is heterogeneous
Sýkora *et al*[16], 2005, Czech Republic and United Kingdom	Human	1+	++	86 symptomatic *H. pylori* positive children	RCT	Two groups - OAC-LC group - Omeprazole, amoxicillin and clarithromycin for 7 d with fermented milk containing *L. casei* DN-114001 for 14 d (n = 39) *vs* OAC group - Omeprazole, amoxicillin and clarithromycin for 7 d (n = 47)	Eradication of *H. pylori*, Endoscopic and Histologic comparison	ITT based eradication rates for the group A were 84.6% and 91.6% by PP analysis. Eradication in the group B was 57.5% in the ITT and 61.3% in the PP group. Eradication success was higher in the group A compared to group B in both ITT (P = 0.0045) and PP analysis (P = 0.0019)
Sheu *et al*[17], 2006, Taiwan	Human	1+	+	138 patients in whom triple therapy failed	RCT	Two groups - yogurt (containing *L. acidophilus La5, Lactobacillus bulgaricus, Bifidobacterium lactis Bb12* and *Streptococcus thermophilus*)-plus-quadruple therapy group for 7 d (n = 69) *vs* quadruple therapy only group (n = 69) for 7 d	Successful eradication of *H. pylori*, drug compliance, side effects	The yogurt-plus-quadruple therapy group had a higher *H. pylori* eradication rate than did the quadruple therapy only group (ITT analysis 85% *vs* 71.1%, P < 0.05; PP analysis- 90.8% *vs* 76.6%, P < 0.05). Side effects were more frequent in the quadruple therapy-only group than in the yogurt-plus-quadruple therapy group
Miki *et al*[20], 2007, Japan	Human	1-	++	69 subjects who were positive for *H. pylori* infection	RCT	Two groups - Fermented milk (*Bifidobacterium bifidum YIT*) (BF-1) (n = 34) *vs* placebo (untreated milk) (n = 35) for 12 wk	Suppressive effect of BF-1 fermented milk on *H. pylori* urease activity and gastric situation	*H. pylori* infection was judged by the C-UBT. *H. pylori*-negativity (below 5%: n = 6 and 4 in the BF-1 and placebo groups, respectively) subjects
Sheu *et al*[18], 2002, Taiwan	Human	1-	+	160 *H. pylori* infected patients	CCT	Two groups - triple plus yogurt (TYG) (containing *L. acidophilus La5, Lactobacillus bulgaricus, Bifidobacterium lactis* Bb12 and *Streptococcus thermophilus*) group (n = 80) *vs* triple only group (TG) (n = 80) for 7 d	Successful eradication of *H. pylori*, drug compliance, side effects	By ITT analysis, the triple-plus-yogurt group had a higher *H. pylori* eradication rate than the triple-only group (P < 0.05) and side effects were more commonly found in the TG than in the TYG. Also a significantly higher proportion of patients in the TYG completed the 7-d regimen than in the TG (67.5% *vs* 43.8%, P < 0.05)
Felley *et al*[21], 2001, Boston	Human	1-	+	53 volunteers infected with *H. pylori*	CCT	Two groups - Acidified milk containing *L. johnsonii* La1 (LC-1) (n = 25) *vs* Placebo (pasteurized milk) (n = 27) for 3 wk followed by 500 mg bid clarithromycin received by all subjects during the last 2 wk	Effect of the given treatment on *H. pylori* density, gastric inflammation and activity	In the LC-1 group, four had higher scores in the antrum, 14 were found to have a decreased *H. pylori* density reflected by lower scores (P = 0.02) and in the placebo group in antrum scores remain identical in 10 volunteers and decreased in 11 (0.08). The results suggest that *H. pylori* infection and gastritis can be down-regulated by LC-1

Cats *et al*[22], 2003, Netherlands	Human	1-	-	14 *H. pylori* positive subjects	CCT	Two groups - Fermented milk (*L.casei*) for 3 wk (n = 14) *vs* control group (n = 6)	Effect of *L.casei* on urease activity *in vivo* (*H. pylori* positive subjects)	Urease activity decreased in nine of the 14 (64%) subjects with *L. casei* supplementation and in two of the six (33%) controls (P = 0.22). A slight, but non-significant, trend towards a suppressive effect of *L. casei* on *H. pylori* *in vivo* may exist
Wang *et al*[19], 2004, Taiwan	Human	1-	-	70 volunteers infected with *H. pylori*	CCT	Two groups - AB yogurt (*containing L. acidophilus* La5, *Lactobacillus bulgaricus*, *Bifidobacterium lactis* Bb12 and *Streptococcus thermophilus*) (n = 59) *vs* milk placebo (n = 11) for 6 wk	Effect of yogurt on *H. pylori* infection in humans	Administration of AB-yogurt decreased the urease activity of *H. pylori* after 6 wk of therapy (P < 0.0001). Regular intake of yogurt containing Bb12 and La5 effectively suppressed *H. pylori* infections in humans
Park *et al*[23], 2001, South Korea	Human	NR	-	40 *H. pylori* infected volunteers	CCT	Two groups - Fermented milk (*Lactobacillus acidophilus*, *Lactobacilus casei*) (n = 21) *vs* Placebo (n = 19) for 4 wk	Eradication of *H. pylori*, Comparison of endoscopic findings, Compliance	All patients were compliant and the *H. pylori* density of antrum tended to decrease in treatment group compared with placebo group (P = 0.072). 3 cases in treatment group were noted for negative conversions of both rapid urease test and C-UBT
Kim *et al*[24], 2007, South Korea	Human	FTNA	FTNA	262 *H. pylori* infected patients	CCT	Two groups - triple plus yogurt group for 3 wk (n = 147) *vs* triple only group (n = 115) for 1 wk	Eradication of *H. pylori*	In PP analysis, *H. pylori* eradication rate in the yogurt group, 87.7% was marginally higher than that in control group, 78.4% (P = 0.055). And according to ITT analysis, the eradication rate in the yogurt group, 78.2% was also marginally higher than that of control group, 69.5% (P = 0.062)
Negative								
Goldman *et al*[25], 2006, Argentina	Human	1+	++	65 children who tested positive for *H. pylori*	RCT	Two groups - triple therapy with probiotic food (commercial yogurt containing *Bifidobacterium animalis* and *Lactobacillus casei*) (n = 33) *vs* triple therapy with placebo (milk fluid) (n = 32)	Eradication of *H. pylori*	We found no significant differences in *H. pylori* eradication rates at 1 and 3 mo between the treated group (ER 45.5% and 42.4%) and the control group (ER = 37.5% and 40.6%). Study could not demonstrate an adjuvant effect of the studied probiotic food to triple therapy in the eradication of *H. pylori* infection in children
Song *et al*[26], 2005, South Korea	Human	NA	-	70 patients with duodenal ulcer	CCT	Two groups - triple-plus-fermented milk (*Lactobacilli*) (n = 35) *vs* triple plus placebo (n = 35)	*H. pylori* eradication rate, Fermented milk group reduces treatment-related adverse reactions	Eradication was successful in 88.6% in the *Lactobacilli* group and 85.7% in the placebo group (P = 1.00). *Lactobacillus* containing fermented milk couldn't exert beneficial effects on *H. pylori* eradication or treatment-related adverse reactions

[1]Levels of evidence: 1++ High quality meta-analysis, systematic reviews of RCTs, or RCTs with a very low risk of bias; 1+ Well conducted meta-analysis, systematic reviews of RCTs or RCTs with a low risk of bias; 1- Meta-analysis, systematic reviews or RCTs or RCTs with a high risk of bias; 2++ High quality systematic reviews of case-control or cohort studies or high quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal; 2+ Well conducted case control or cohort studies with a low risk of confounding, bias, or chance and a significant risk that the relationship is not causal; 3 Non-analytic studies, *e.g.*, case reports, case series; 4 Expert opinion. [2]Quality rating for individual studies: ++ Applies if all or most criteria from the checklist are fulfilled; where criteria are not fulfilled the conclusions of the study or review are thought very unlikely to alter; + Applies if some of the criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought unlikely to alter; - Applies if few or no criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought likely or very likely to alter. *H. pylori*: *Helicobacter pylori*; RCT: Randomised controlled trial; CCT: Controlled clinical trial; CT: Clinical trial; C-UBT: ^{13}C-urea breath test; FMPP: Fermented milk based probiotic preparation; NR: Not reported; FTNA: Full text not available; NS: Not significant; LC-1: *L. johnsonii* La1.

ments (Tables 1, 2 and 3). First, if whey proteins have clinically significant anti-Helicobacter properties then FMPP alone or in combination with standard therapy should have documented effectiveness (improvement in eradication rates)[15-26]. Secondly, capsule based probiotic preparations (bacteria only) should be partly or completely ineffective in *H. pylori* eradication[27-38]. Thirdly, if FMPP's are compared with a fermented milk control group then in the control group there should be some improvement partly or completely negating the effect of the addition of bacteria in the treatment group[39-41].

As summarized in Tables 1-3, the available evidence supports the above assertions and arguments. It is evident from the clinical studies and meta-analysis presented in Tables 1-3 that FMPPs have some efficacy against *Helicobacter* (10 positive trials and one positive meta-anal-

Table 2 Studies comparing capsule based probiotic (bacteria only) with placebo or standard therapy plus capsule based probiotic *vs* standard therapy

Ref.	Type of trial	Evidence grade[1]	Quality rating[2]	Subjects	Study design	Study groups/ methods	Outcome variable/s	Results and conclusions
Positive								
Canducci *et al*[27], Italy, 2000	Human	1+	+	120 *H. pylori* positive patients	RCT	Two groups: RCA (Rabeprazole, Clarithromycin, Amoxycillin) group- triple therapy (n = 60), RCAL group- triple therapy with Lactéol Fort for 7 d	Effect of *L. acidophilus* could improve the efficacy of a standard anti-*H. pylori* therapy	In RCA group eradication was successful in 72% at PP analysis or 70% at ITT analysis and in RCAL group eradication was achieved with 88% with PP analysis, 87% with ITT analysis
Negative								
Gotteland *et al*[28], 2005	Human	1+	+	254 children positive for *H. pylori*	RCT	Three groups: Antibiotics (group Ab)- (n = 57) for 8 d, *Lactobacillus acidophilus* LB (group Ab)- (n = 63) for 8 wk, *Saccharomyces boulardii* plus inulin (group Sb1)- (n = 62) 8 wk	To evaluate the capacity of *Lactobacillus acidophilus* LB and of symbiotic combination of Sb plus inulin to interfere with *H. pylori* colonization in children	*H. pylori* was eradicated in 66%, 12% and 6.5% of the children from the Ab, Sb1 and LB groups, respectively. A moderate but significant difference in Δ DOB was detected in children receiving living Sb1, but not in those receiving LB
Lionetti *et al*[29], 2006, Italy	Human	1+	++	40 *H. pylori* positive children	RCT	Two groups: Group A- 10 d sequential therapy plus *L. reuteri* ATCC 55730, Group B-Placebo with the same therapy	Effect of *Lactobacillus reuteri* to prevent or minimize the gastrointestinal side-effects	No significant differences were observed between the groups in the success of *H. pylori* eradication. Treatment was successful in 17 of 20 [85% (95%CI: 68-100)] patients in probiotic supplemented when compared with 16 of 20 patients in placebo group [80% (95%CI: 61–99)] (P = NS)
Nista *et al*[30], 2004, Italy	Human	1+	++	106 *H. pylori* positive patients	RCT	Two groups: Group A- triple therapy for 7 d plus *Bacillus clausii* (probiotic) for 14 d starting from the first day of the treatment (n = 54) Group B- triple therapy plus placebo (n = 52)	Effect of probiotic on incidence and severity of antibiotic-associated side-effects during anti- *H. pylori* therapy and eradication was evaluated with means of ^{13}C-urea breath test	The *H. pylori* eradication rate was similar between *B. B. clausii* and placebo groups. In particular, ITT analysis has shown *H. pylori* was eradicated in 39 of 54 patients (72.2%) in the *B. clausii* group and in 37 of 52 patients (71.15%) in the placebo group. In PP population, *H. pylori* was eradicated in 39 of 50 patients (78%) in the B. clausii group and in 37 of 50 patients (74%) in the placebo group
Myllyluoma *et al*[31], 2005, Finland	Human	1+	+	47 subjects with *H. pylori* infection	CCT	Two groups: Group A –probiotic drink (n = 23), group B- Placebo (n = 24) during *H. pylori* eradication and for 3 wk following the treatment	Effect of probiotic therapy on symptoms associated with the recommended *H. pylori* eradication treatment. As a secondary endpoint to find out whether this therapy could improve the eradication rate	The *H. pylori* eradication rate was non-significantly higher in the group receiving probiotic therapy (91% *vs* 79%, P = 0.42)
Cindoruk *et al*[32], 2007, Turkey	Human	1+	+	124 patients with *H. pylori* infection	RCT	Two groups: Group A- triple therapy plus *S. boulardii*, Group B- triple therapy plus placebo for 14 d	Efficacy and safety of *S. boulardii* in the prevention of side effects and the eradication success of anti-*H. pylori* therapy	*H. pylori* eradication rate, although higher in the treatment group, was statistically similar in treatment and control groups: 71% (44/62) *vs* 59.7% (37/62), respectively (P > 0.05)

Armuzzi *et al*[33], 2001, Italy	Human	1+	+	60 healthy asymptomatic subjects screened positive for *H. pylori* infection	CCT	Two groups: Group A- triple therapy for 7 d plus *Lactobacillus* GG for 14 d during and the week after eradication therapy, Group B- triple therapy plus placebo	Effect of probiotic *Lactobacillus* GG to minimize or to prevent the occurrence of gastrointestinal side effects	*H. pylori* eradication rates in group A was 83.33% (25/30) and in group B was 80% (24/30). *H. pylori* eradication rate had no significant difference
Guo *et al*[34], China, 2004	Human	FT NA	FT NA	97 *H. pylori* positive symptomatic patients	CCT	Two groups: treatment group (triple therapy plus Bifid triple viable capsule containing *Bifidobacteria longum*, faecal streptococci, *Lactobacillus acidophilus*) (n = 47) control group: triple therapy (n = 50)	Efficacy of probiotic in the treatment of *H. pylori*	Eradication rate was 93.6% (44/47) in treatment group and 88% in control group (44/50). *H. pylori* eradication rate had no significant difference
Armuzzi *et al*[35], 2001, Italy	Human	FT NA	FT NA	120 healthy asymptomatic subjects screened positive for *H. pylori* infection	CCT	Two groups: Group A- triple therapy for 7 d plus *Lactobacillus* GG for 14 d during and the week after eradication therapy, Group B- triple therapy plus placebo	Effect of probiotic *Lactobacillus* GG to minimize or to prevent the occurrence of gastrointestinal side effects.	*H. pylori* eradication rates in group A was 80% (48/60) and in group B was 76.67% (46/60). *H. pylori* eradication rate had no significant difference
Cremonini *et al*[36], Italy, 2002	Human	FT NA	FT NA	85 *H. pylori* positive, asymptomatic patients	CCT	Four groups- received both during and for 7 d after a 1 wk-triple therapy Group Ⅰ- *Lactobacillus* GG (n = 21), group Ⅱ-*Saccharomyces* boulardii (n = 22), group Ⅲ-*lactobacillus* spp. And bifidobacteria (n = 21), group Ⅳ-placebo (n = 21)	Efficacy of probiotic in the eradication of *H. pylori* infection	The *H. pylori* eradication rate was almost identical between the probiotic and placebo groups
Tursi *et al*[37], 2004, Italy	Human	FT NA	FT NA	70 patients with persistent *H. pylori* infection	CCT	Two groups- group A- quadruple therapy plus bacteria *lactobacillus casei* subsp. casei DG or group B- quadruple therapy only	Effect of probiotic supplementation on the effectiveness and tolerability of a new second-line 10 d quadruple therapy	*H. pylori* was negative in 33/34 group A patients (PP: 97.05% ITT: 94.28%) and 30/32 Group B patients
Cao *et al*[38], China, 2005	Human	FT NA	FT NA	128 *H. pylori* positive symptomatic patients	CCT	Two groups: Group A -quadruple therapy plus *Clostridium butyricum* group B- quadruple therapy	Effect of treatment given in eradication of *H. pylori*	Eradication rates in group A 96.88% (62/64) and group B 92.19% (59/64) was not significantly different

[1]Levels of evidence: 1++ High quality meta-analysis, systematic reviews of RCTs, or RCTs with a very low risk of bias; 1+ Well conducted meta-analysis, systematic reviews of RCTs or RCTs with a low risk of bias; 1- Meta-analysis, systematic reviews or RCTs or RCTs with a high risk of bias; 2++ High quality systematic reviews of case-control or cohort studies or high quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal; 2+ Well conducted case control or cohort studoes with a low risk of confounding, bias, or chance and a significant risk that the relationship is not causal; 3 Non-analytic studies, *e.g.*, case reports, case series; 4 Expert opinion. [2]Quality rating for individual studies: ++ Applies if all or most criteria from the checklist are fulfilled; where criteria are not fulfilled the conclusions of the study or review are thought very unlikely to alter; + Applies if some of the criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought unlikely to alter; - Applies if few or no criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought likely or very likely to alter. *H. pylori*: *Helicobacter pylori;* RCT: Randomised controlled trial; CCT: Controlled clinical trial; CT: Clinical trial; NR: Not reported; NS: Not significant.

ysis compared with 2 negative trials; Argument 1 above). It is also apparent from Table 1-3 that studies using capsule-based probiotic preparations are predominantly negative (1 positive trial compared with 11 showing no benefit; and Argument 2). In support of Argument 3 the overall data on the beneficial effect of bacterial probiotic

Table 3 Clinical trials comparing fermented milk based probiotic preparations *vs* plain fermented milk

Ref.	Type of trial	Evidence grade[1]	Quality rating[2]	Subjects	Study design	Study groups/ methods	Outcome variable/s	Results and conclusions	
Positive									
Pantoflickova *et al*[39], 2003, Switzerland	Human	1-	++	50 *H. pylori* positive healthy volunteers	RCT	Two groups- fermented milk with LC (n = 25) *vs* fermented milk as Placebo (n = 25). Subjects took the treatment twice daily during the first 3 wk and once daily for the next 13 wk	Effect of LC[1] intake without antibiotics on *H. pylori* gastritis, *H. pylori* density	LC[1] intake had a favorable, albeit weak, effect on *H. pylori* associated gastritis, particularly in the antrum. Regular ingestion of fermented milk containing *L.johnsonii* may reduce the risk of developing disorders associated with high degrees of gastric inflammation and mucus depletion	Placebo intake led to a decrease in severity and activity of gastritis in the antrum (inflammatory cell score after 3-wk and 16 wk consumption: 6.3 ± 0.7 and 6.4 ± 1.0, respectively). In the placebo group, mucus depletion scores remained at the same level during the whole duration of the study. *H. pylori* density decreased in 38% of subjects after 3 wk and 50% after 16 wk
Horie *et al*[40], 2004, Japan, South Korea, Egypt	Human	1-	-	42 subjects with *H. pylori* infection	CCT	Two groups- A- test group (yogurt containing 1, 5 g of egg yolk IgY-urease 3 times daily) (n = 22), B- control group (IgY-urease free yogurt) (n = 20)	Effect of IgY-Urease drinking yogurt on C-UBT values	TG showed a reduction in UBT values from 51.18 ± 3.40 at wk 0 to 33.70 ± 3.50 and 31.03 ± 3.54 at 2 and 4 wk resp. Suppression of *H. pylori* infection in humans could be achieved by consumption of drinking yogurt fortified with IgY-urease	CG showed some decrease in UBT values from 51.40 ± 4.48 to 44.38 ± 5.17 and 43.53 ± 5.48 at 0, 2 and 4 wk, resp. There was no significant difference obtained at week 0 and weeks 2 or 4
Sakamoto *et al*[41], 2001, Japan	Human	2-		31 subjects infected with *H. pylori* infection	CT	The study was conducted in two parts. 1st part = 90 g of yogurt (0-9 wk). 2nd part = 90 g yogurt containing LG21 (9-18 wk)	Efficacy of *Lactobacillus gasseri* OLL2716 (LG21) as a probiotic for *Helicobacter pylori*	The [13C] urea breath test and assays of serum pepsinogens revealed a significant improvement following LG21 treatment. LG21 was thus determined to be effective in both suppressing *H. pylori* and reducing gastric mucosal inflammation	There was no significant difference in C-UBT levels at 0 (26.2 ± 15.1) and 9 (26.6 ± 13.7) wk

[1]Levels of evidence: 1++ High quality meta-analysis, systematic reviews of RCTs, or RCTs with a very low risk of bias; 1+ Well conducted meta-analysis, systematic reviews of RCTs or RCTs with a low risk of bias; 1- Meta-analysis, systematic reviews or RCTs or RCTs with a high risk of bias; 2++ High quality systematic reviews of case-control or cohort studies or high quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal; 2+ Well conducted case control or cohort studoes with a low risk of confounding, bias, or chance and a significant risk that the relationship is not causal; 3 Non-analytic studies, *e.g.*, case reports, case series; 4 Expert opinion. [2]Quality rating for individual studies: ++ Applies if all or most criteria from the checklist are fulfilled; where criteria are not fulfilled the conclusions of the study or review are thought very unlikely to alter; + Applies if some of the criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought unlikely to alter; - Applies if few or no criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought likely or very likely to alter. *H. pylori*: *Helicobacter pylori;* RCT: Randomised controlled trial; CCT: Controlled clinical trial; CT: Clinical trial; C-UBT: ^{13}C-urea breath test.

preparations in Helicobacter eradication can, at best, be classified as "equivocal" (3 trials with weak methodology and equivocal results). This apprehension is further substantiated by a meta-analytic sub-analysis presented in an earlier report[42]. In this sub-analysis the beneficial effect of these preparations was minimal and it failed on exclusion sensitivity analysis (exclusion of one study majorly altered results) in consonance with the hypothesized argument.

In the context of studies comparing FMPP with fermented milk, several results are noteworthy. Of the three trials reporting control group data, two (one RCT and one CCT; Evidence grade 1-)[39,40] documented an improvement in gastritis or C-UBT values in the control group which is consistent with the argument presented earlier. In the third pre- and post-intervention trial (clinical trial, evidence grade 2[41]) no significant differences were observed during the period that yogurt was administered alone. Hence, although there are some discrepant results the preponderance of the available evidence appears con-

Table 4 Whey protein components and its basic properties

Whey components	Concentration (g/L)	% of Whey Protein	Molecular weight (kDa)	Number of amino acids residues	Biological properties	Recommendation grade against Helicobacter[1]
β-Lactoglobulin	1.3	50%-55%	18277	162	Source of essential and branched chain amino acids	-
α-Lactalbumin	1.2	20%-25%	14175	123	Primary protein found in human breast milk Source of essential and branched chain amino acids	D
Immunoglobulins (A, B and C)	0.7	10%-15%	25000 (light chain) + 50000-70000 (heavy chain)	-	Primary protein found in colostrum Immune modulating benefits	D
Lactoferrin	0.1	1%-2%	80000	700	Antioxidant Antibacterial, antiviral, and antifungal Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood, and mucus	A
Lactoperoxidase	0.03	0.50%	70000	612	Inhibits growth of bacteria	-
Bovine Serum Albumin	0.4	5%-10%	66267	582	Source of essential amino acids Large protein	-
Glycomacropeptide	1.2	10%-15%	6700	64	Source of branched chain amino acids Lacks the aromatic amino acids phenylalanine, tryptophan and tyrosine	D

[1]Grades of recommendations: A: At least one meta-analysis, systematic review, or RCT rated as 1++ and directly applicable to the target population or A systematic review of RCTs or a body of evidence consisting principally of studies rated as 1++ directly applicable to the target population and demonstrating overall consistency of results; B: A body of evidence including studies rated as 2++ directly applicable to the target population and demonstrating overall consistency of results or Extrapolated evidence from studies rated as 1++ or 1+; C: A body of evidence including studies rated as 2+ directly applicable to the target population and demonstrating overall consistency of results or Extrapolated evidence from studies rated as 2++; D: Evidence level 3 or 4 or Extrapolated evidence from studies rated as 2+.

sistent with the hypothesis that whey milk proteins may partly or completely explain the anti-Helicobacter properties of fermented milk based probiotic preparations.

Overall, the recommendation for fermented milk may be classified as Recommendation Grade-A. The magnitude of the benefit achieved by FMPPs is small (10%) but holds across a variety of preparations. FMPPs also carry the potential inherent advantage of better patient acceptability. Thus, they could offer a viable alternative for complementing traditional regimens. Further research is necessary to identify the active substrate/s and to define the exact product to be used, the optimal clinical setting (prevention/treatment, first line therapy/recurrence, symptomatic/asymptomatic, gastritis/ulcer diseases, treatment failure *etc.*) and potential benefits in the setting of high antibiotic resistance.

WHEY PROTEINS

Whey proteins are globular water soluble molecules constituting 20% of the milk protein system. The whey protein profile, including general chemical, physicochemical and biological properties is depicted in Table 4. β-LG comprises the maximum percentage of whey protein but it has not been documented to possess any anti-bacterial properties. Other proteins have promising antibacterial

attributes and hence have been studied in *in vitro*, *in vivo* and in human trials. With specific reference to *H. pylori* infection and associated conditions lactoferrin, α-LA, glycomacropeptide and immunoglobulins appear to be potentially relevant and warrant further discussion.

Bovine lactoferrin

Bovine lactoferrin, an iron-binding glycoprotein, is a non-enzymatic antioxidant found in the whey fraction of fermented milk as well as in colostrum. The possibility that bLF may help to improve the *H. pylori* eradication rate was first conceived in 1997 when, in an *in vitro* study by Yamazaki *et al*[43], bLf was found to be bactericidal to *H. pylori* in Brucella broth. Later *in vitro* studies have confirmed the same and yielded evidence of the possible mechanism of bactericidal action of bLf relating it to the high iron-binding affinity and prevention of iron utilization by *H. pylori*[44,45]. An additional mechanism based on the interaction of bLf with the bacterial surface is also suggested in the context of bactericidal effect on *S. mutans* and *V. cholerae*[46]. It has been observed that bLf can bind to the outer membrane of Gram-negative bacteria and trigger the release of lipopolysaccharides, and kill the bacteria through osmotic damage[47,48]. Building on the available evidence Wada *et al*[49], in their study, examined the therapeutic effect of bLf on *H. pylori* infection using *in vitro* and *in vivo* experimental systems. In the experiment a significant inhibition of *H. pylori* binding to gastric epithelium was accomplished within 8 h after incubation. As a follow up experiment mice infected with *H. pylori* were given 10 mg of bLf orally every day and their stomachs were removed after 2 wk. 40.0% of all *H. pylori* attached themselves to the epithelium in the stomach of the untreated mice, whereas only 19.9% of the *H. pylori* did in the bLf-treated mice. However, in a similar experiment by Huynh *et al*[50], bLF, desferrioxamine and human recombinant lactoferrin had positive *in vitro* effects but all three failed to reduce *H. pylori* load in mice.

The above experimental evidence led to several human clinical trials. These are summarized in Table 5[43,51-57]. As presented, 5 (of 7 available) positive clinical trials and a meta-analysis appear to establish the beneficial effect of bLf (4%-17% as per meta-analysis) on *H. pylori* eradication fairly well[58]. The positive response was variously explained by the authors: (1) synergistic action of the antibiotics with bLf against *H. pylori*; (2) Inhibition of *Helicobacter* growth in an acidic pH by bLf; (3) Ability of bLf to bind to iron inhibiting growth of *H. pylori*; and (4) decrease in incidence of side effects and non-compliance. Two studies by Zullo *et al*[56] and Imoto *et al*[57] did not show any significant difference on addition of lactoferrin to triple therapy. In the first study this could be explained by the lack of synergism between lactoferrin and amoxicillin[56]. Alternatively, the anti-bacterial effect of lactoferrin based on bacterial membrane damage of Gram negative bacteria could be marginalized when amoxicillin is administered. In the second study the authors using quadruple therapy (rabeprazole, clarithromycin, tinidazole and lactoferrin) showed a statistically insignificant improvement in the eradication rate (4% in ITT analysis and 7% in per-protocol analysis). The results of this trial are limited by marked geographical heterogeneity (multicentre trial) in eradication rates.

Although the available evidence suggests that bLf is beneficial (Recommendation Grade-A), the magnitude of the documented benefit is small. Given that it lacks the inherent advantage in patient acceptability (requires to be given as a drug), the concept that fermented milk potentially has a clinically significant benefit (other than suggesting that whey protein may be partly/completely responsible for the benefit with FMPP) remains unclear. Its role in various clinical settings and more so in the presence of high antibiotic resistance deserves further exploration.

α-LA

α-LA is a major milk protein comprising 20-25% of whey proteins and has strong calcium binding ability. α-LA is reported to be biologically active *in vivo* with well-demonstrated antiulcer activity in rats. Matsumoto *et al*[59], in an *in vivo* study using ethanol ulcer model rats, documented 82% reduction of ulcerative lesion index using 200 mg/kg bw of α-LA. Similar results were reported by Mezzaroba *et al*[60], with absolute alcohol and indomethacin ulcer model rats given commercial α-LA. This intervention resulted in 30%-70% reduction in the ulcerative lesion index in comparison with controls. The exact mechanism of the protective effect and its impact on *Helicobacter* is not well studied. However, as reported, whey protein concentrates have consistently shown anti-Helicobacter properties. The minimal evidence on the subject precludes any definitive comment on the potential of α-LA as an anti-Helicobacter agent. The paucity of literature on the subject presents wide scope for future research.

Glycomacropeptide

Glycomacropeptide (GMP), also referred to as caseinomacropeptide and caseinoglycopeptide, is formed when bovine κ-casein is hydrolysed into para-κ-casein, which remains with the curd, and GMP, which is removed with the whey. It constitutes 15%-20% of whey protein. GMP has also been found to have several immunomodulatory functions and antibacterial properties. Otani *et al*[61] demonstrated that GMP, which contains sialic acid, inhibits the activity of *Salmonella typhimurium* lipopolysaccharide, inhibiting bacterial and viral adhesion especially to epithelial cells and dental plaque[62,63]. Other relevant properties like suppression of gastric secretions in dogs have been reported by a study group[64].

A study done in Japan attempted to enhance the ability of glycopeptides to bind pathogenic bacteria *in vivo* by conjugating with the non-digestible saccharides. The results of this study suggest that GMP could be a promising agent for preventing intestinal infection using its ability to bind pathogenic bacteria[65]. In the context

Table 5 Studies comparing bovine lactoferrin with placebo or "standard therapy + bovine lactoferrin" with "standard therapy"

Ref.	Type of trial	Evidence grade[1]	Quality rating[2]	Subjects	Study design	Study groups	Outcome variable	Results and conclusion
Sachdeva *et al*[58], 2009, India	Metaanalysis	1+	++	5 trials; 682 subjects [bLF group (n = 316); control group (n = 366)]	Metaanalysis of human RCTs/CCTs	Trials had to be randomized or quasi-randomized and controlled, using bLF in the intervention group treating *Helicobacter*-infected patients. The only difference between the two groups had to be bLF	Eradication of *H. pylori*; adverse events of eradication therapy	The pooled odds ratio (5-studies) for eradication by intention to treat analysis was 2.22 (95%CI: 1.44-3.44; P = 0.0003) using the fixed effects model (FEM) and 2.24 (95%CI: 1.15-4.35; P = 0.0003) using the random effects model (REM) (Cochran's Q = 6.83; P = 0.145). The pooled risk difference was 0.11 (95%CI: 0.05 -0.16; P = 0.0001) by FEM (Cochran's Q = 6.67; P = 0.154) and 0.10 (95%CI: 0.04-0.17; P = 0.0023) by REM. There was no significant difference in incidence of adverse effects
Di Mario *et al*[51], 2003, Italy	Human	1+	+	150 consecutive *H. pylori*-positive patients suffering from dyspeptic symptoms, gastritis and peptic ulcer disease	RCT	Three groups – A-triple therapy (rabeprazole,clarithromycin,tinidazole) with lactoferrin for 7 d (n = 51), B-triple therapy for 7 d (n = 52), C- triple therapy for 10 d (n = 47)	Efficacy of standard triple therapy plus bovine lactoferrin in the eradication of *H. pylori*	Eradication rates (ITT) were A-92.2%, B-71.2%, C-70.2 %. Results suggest that lactoferrin tested in the present study was effective in curing *H. pylori* and could be a new agent to assist the antimicrobials in the eradication of the bacterium
Di Mario *et al*[52], 2006, Italy	Human	1+	+	402 consecutive *H. pylori*-positive patients suffering from dyspeptic symptoms, gastritis and peptic ulcer disease	RCT	Three groups – A- triple therapy (esomeprazole, clarithromycin ,tinidazole) for 7 d (n = 136), B-lactoferrin followed by triple therapy for 7 d (n = 132), C- triple therapy with lactoferrin (n = 134)	Efficacy of bovine lactoferrin in the treatment of *H. pylori* infection	Eradication rate (ITT)- A- 77%, B-73%, C = 90%. Incidence of side effects was A- 9.5%, B- 9%, C- 8.2% Results demonstrate that bovine lactoferrin is an effective adjuvant to triple therapy for eradication of *H. pylori* Infection
Okuda *et al*[53], 2005, Japan	Human	1-	+	59 *H. pylori* infected healthy volunteers or children who were enrolled in a previous epidemiological study	CCT	Two groups- bLF (n = 31), placebo (n = 28)	Efficacy of a single administration of bLF. Improvement of *H. pylori* infection, adverse effects	Positive response (> 50% decrease in C-UBT values) was observed in 10 of 31 bLF-treated subjects and 1 of 28 control subjects, indicating that the rate of positive response in the bLF group was significantly higher than that in the control group
Tursi *et al*[54], 2007, Italy	Human	1-	+	70 consecutive patients with persistent *H. pylori* infection after failure of a first standard treatment	CCT	Two groups- A-quadruple therapy (ranitidine bismuth citrate plus triple therapy-esomeprazole ,amoxicillin, tinidazole) (n = 35), B- quadruple therapy plus lactoferrin (n = 35)	Efficacy and tolerability of bLF supplementation to this quadruple therapy in re-treating *H. pylori* infection	Eradication rate- A-88.57%, B-94.28%. Side effects- A-29.41%, B-17.64%. bLF supplementation was found effective in reducing side-effect incidence. It seems capable of achieving a slight (NS statistically) improvement in eradicating *H. pylori*

Zullo *et al*[55], 2005, Italy	Human	1+	++	133 consecutive patients with non-ulcer dyspepsia and *H. pylori* infection	RCT	Two groups- A- triple therapy for 7 d (n = 68), B- quadruple therapy (triple therapy plus lactoferrin) (n = 65)	Eradication rate of *H. pylori* infection, side effects and compliance	Eradication rate (ITT) A- 77.9%, B- 76.9%. Side effects- A –10.3%, B- 9.2%. Quadruple therapy with bLF did not significantly increase the *H. pylori* cure rate of standard 7-d clarithromycin-amoxycillin based triple therapy in non-ulcer dyspepsia patients
Zullo *et al*[56], 2007, Italy	Human	1+	+	144 consecutive dyspeptic patients	RCT	Two groups – A- triple therapy (rabeprazole, levofoxacin, amoxycillin) (n = 72), B- quadruple therapy (rabeprazole, clarithromycin, tinidazole plus bovine lactoferrin) (n = 72)	Eradication rate of *H. pylori* infection, side effects and compliance	Eradication rate (ITT) A- 68.1%, B-72.2%. *H. pylori* eradication rate following both quadruple therapy with lactoferrin and a low-dose PPI, triple therapy with levofloxacin is disappointingly low
Imoto *et al*[57], 2004	Human	FTNA	FTNA	25 *H. pylori* positive healthy volunteers	CCT	Two groups- A- bLf mixed with a commercial yogurt (n = 16) B- yogurt (n = 9)	Effect of bLf against *H. pylori*	The C-UBT values at week 8 were significantly lower than those at week 0 in the bLf group (P < 0.01), whereas no difference was observed in the control group

[1]Levels of evidence: 1++ High quality meta-analysis, systematic reviews of RCTs, or RCTs with a very low risk of bias; 1+ Well conducted meta-analysis, systematic reviews of RCTs or RCTs with a low risk of bias; 1- Meta-analysis, systematic reviews or RCTs or RCTs with a high risk of bias; 2++ High quality systematic reviews of case-control or cohort studies *or* High quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal; 2+ well conducted case control or cohort studoes with a low risk of confounding, bias, or chance and a significant risk that the relationship is not causal; 3 Non-analytic studies, eg case reports, case series; 4 Expert opinion. [2]Quality rating for individual studies: ++ Applies if all or most criteria from the checklist are fulfilled; where criteria are not fulfilled the conclusions of the study or review are thought very unlikely to alter; + Applies if some of the criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought unlikely to alter; - Applies if few or no criteria from the checklist are fulfilled; where criteria are not fulfilled or are not adequately described, the conclusions of the study or review are thought likely or very likely to alter. *H. pylori*: *Helicobacter pylori*; RCT: Randomised controlled trial; CCT: Controlled clinical trial; CT: Clinical trial; C-UBT: ^{13}C-urea breath test; FTNA: Full text not available; NS: Not significant.

of *Helicobacter* infection several authors have expressed the view that GMP has gastroprotective properties[66] but there is no direct evidence supporting its role in its eradication. Currently, in the absence of direct evidence the potential benefit of GMP in the treatment of *H. pylori* infection remains speculative.

Immunoglobulins

Immunoglobulins constitute a complex group, the elements of which are produced by B-lymphocytes. They make a significant contribution to the whey protein content (10-15%). Some of them attach to surfaces, where they behave as receptors, whereas others function as antibodies, which are released in the blood and lymph. Early *et al*[67], in an *in vitro* study, demonstrated that whey protein concentrates produced using milk from *H. pylori* immunized cows contain antibodies that are active at the pH of the stomach, and bactericidal against *H. pylori in vitro*. Oona *et al*[68], in their study on 20 children suffering from recurrent abdominal pain and with proven *H. pylori* infection, showed alleviation of gastritis and/or a decrease in the degree of colonization of the antrum mucosa in 9/14 children, and of the corpus mucosa in 7/15 children using immune colostrum of cows immunized (whole-cell vaccine prepared with *H. pylori* strain NCTC 11637) before calving. It is clear that evidence on the *in vivo* effects of the immunoglobulin in prevention or treatment of *H. pylori* infections in humans is only suggestive and deserves further work.

CONCLUSION

In conclusion, FMPP and bovine lactoferrin appear to be beneficial in *Helicobacter* eradication (Evidence Grade-A or -B in various settings with level 1++ studies available). Evidence for α-lactabumin and whey protein concentrates enriched in immunoglobulins is "suggestive of benefit". However the studies are small and/or based on animals (level 3 or 4 studies only; no grading possible). Literature on glycomacropeptide is very preliminary precluding relevant inferences. No studies directly comparing the efficacy of individual components amongst themselves or to FMPP were available. Overall, the magnitude of the potential benefit documented so far for the group is small and the precise clinical settings are poorly defined. This restricts more widespread use of this group as a complementary therapy in a nutraceutical setting hinging on better patient acceptability/compliance. Further work is necessary to identify the optimal substrate, fermentation process, dose of administration and the ideal clinical setting (prevention/treatment, first line therapy/recurrence, symptomatic/asymptomatic, gastri-

tis/ulcer diseases *etc.*). The potential of this group in high antibiotic resistance or treatment failure settings presents interesting possibilities and deserves further exploration.

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P- Reviewers: Bugaj AM, De Re V, Jonaitis L, Koulaouzidis A, Mohammadi M, Nakajima H, Tovey FI
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ISSN 1007-9327
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