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ORIGINAL ARTICLE

Impact of a two-bacterial-strain formula, containing *Bifidobacterium animalis lactis* BB-12 and *Enterococcus faecium* L3, administered before and after therapy for *Helicobacter pylori* eradication

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ABSTRACT

BACKGROUND: Results from the meta-analysis of randomized controlled studies demonstrate that the adjuvant use of probiotics enhances the rate of *Helicobacter pylori* (Hp) eradication, reducing concomitantly the occurrence of side effects, mainly diarrhea. *Bifidobacterium animalis lactis* BB-12 and *Enterococcus faecium* L3 are two bacterial strains reported to clinically improve the rate of Hp eradication, reducing concomitantly the occurrence of side effects.
METHODS: Due to our pragmatic and routine use of these two strains as an adjuvant therapy to antibiotics and proton pump inhibitors (PPIs) during attempted Hp eradication, we have analyzed retrospectively their impact on the outcome.
RESULTS: Our results, obtained through a highly pragmatic clinical approach, demonstrate that the probiotic add-on therapy before and after the triple or quadruple therapy to eradicate Hp increases eradication rates and reduces side effects. Moreover, even if observed in only a small cohort of patients, the treatment seems to improve the eubiosis of the gut microbial consortium.
CONCLUSIONS: Administration of BB-12 and L3 strains as an adjuvant regimen during Hp eradication therapy has better success than conventional therapy (antibiotics plus a PPI) alone.

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KEY WORDS: Antibacterial agents; Proton pump inhibitors; Probiotics; Disease eradication; Diarrhea; *Helicobacter pylori*.

Indications for *Helicobacter pylori* (Hp) eradication therapy include peptic ulcer disease, low-grade gastric mucosa-associated lymphoid tissue lymphoma, atrophic gastritis, and after resection of early gastric cancer. Furthermore, eradication therapy is considered an option in functional dyspepsia, in first-degree relatives of gastric cancer patients, or before starting long-term therapy with nonsteroidal anti-inflammatory drugs or acetylsalicylic acid.[1] Amoxicillin, or metronidazole or tinidazole, and clarithromycin plus a proton pump inhibitor (PPI), well known as a 7-day concomitant triple therapy, have long been considered the first-line choice for Hp eradication. Some researchers propose that this regimen should be avoided owing to increasing resistance and still having a failure rate of up to 30%.[2-4] A 10-day concomitant therapy and 10-day sequential therapy have been reported to obtain better results.[5] In most regions of the world, a 4-drug treatment, also known as quadruple therapy, consisting of a PPI, bismuth, metronidazole or tinidazole, and tetracycline provides the best results,

with the rate for lack of eradication lying between 10% and 20%.[6] However, the quadruple therapy is considered somewhat more aggressive, causing more severe side effects, and some patients do not seem to be the right candidates for this therapy, at least as a first-line therapy.[7]
Therefore, the application of adjuvant approaches to improving the efficacy of triple (sequential or concomitant) and quadruple therapy, reducing at the same time – as much as possible – the incidence of side effects, is considered clinically important. Results from the meta-analysis of randomized controlled studies demonstrate that the adjuvant use of probiotics enhances the rate of Hp eradication, reducing concomitantly the occurrence of side effects, mainly diarrhea.[8-10] From the second half of 2017, in order to improve the rate of Hp eradication and to reduce the occurrence of side effects, we have used in our routine practice a nutraceutical product containing both *Bifidobacterium animalis lactis* BB-12 and *Enterococcus faecium* L3. Both of these probiotic strains have indeed been clinically described to have an anti-Hp effect, while the BB-12 strain has also been shown to reduce diarrhea episodes in subjects treated with antibiotics and a PPI to eradicate Hp.[11-16] This study is therefore the retrospective analysis of the results that we have obtained from Hp treatment between June 2017 and May 2019 by administering, in addition to PPI and antibiotic therapy, these two strains for 14 days before and 4 weeks after the use of conventional therapies.[17]

Materials and methods

Study design and aim

Our study corresponds to a retrospective analysis of the data obtained from our pragmatic and routine procedures conducted at the Digestive Endoscopy Dept. at Ceva Hospital (Ceva, Cuneo, Italy) between June 2017 and May 2019. The aim of the study was to investigate the role played by a nutraceutical product containing *Bifidobacterium animalis lactis* BB-12 and *Enterococcus faecium* L3 as an adjuvant to the therapy for eradicating Hp. All patient data were completely anonymized and the study was performed in accordance with the ethical standards established by the Declaration of Helsinki and by the local institutional committee. Despite the retrospective and anonymized features of the study, all patients provided signed informed consent to publish the results.

Patient selection and Hp detection

Subjects aged over 18 eligible for our study were patients with persistent gastroduodenal symptoms testing positive for Hp infection. Detection was always carried out by an endoscopic procedure, with biopsies of gastric tissue for histological assessment. To validate Hp eradication, 8 weeks after the last PPI dose, we used either the urea breath test or fecal antigen tests (primarily the latter). The following patients were not included in our retrospective analysis: those who had ingested bismuth salts, PPIs, or antibiotics in the previous 8 weeks; those with a known allergy to penicillin (or to antibiotics in general); and those who had undergone previous gastrointestinal surgery. Patients with a past history of gastric malignancy were also excluded.

Treatment scheme

Each patient, according to his/her own medical history and physician opinion, was treated to eradicate Hp either with a 10-day sequential scheme (5 days with a PPI, twice a day, along with 1 g of amoxicillin, twice a day also, followed by 5 days, with PPI as described before, with clarithromycin 500 mg, twice a day, along with tinidazole 500 mg, twice a day) with or without a probiotic product (2 weeks before and 4 weeks after the sequential scheme described above, at a dose of 1 sachet mid-morning) or with a 10-day treatment with a PPI, twice a day, along with bismuth (140 mg × 12/day), tetracycline (125 mg × 12/day), and metronidazole (125 mg × 12/day) with or without the same dose of the probiotic product described above. The add-on therapy with probiotics was suggested by the physician to all patients, especially to those demonstrating anxiety about the therapy, or fear of the possible side effects, or who experienced other gastrointestinal symptoms, such as persistent mushy fecal consistency or considerable digestive difficulties. In any case, only approximately 50% of patients agreed to the additional probiotic treatment, probably because of the long duration (6 weeks), or the cost of the proposed regimen. It was recommended that the probiotic treatment run for 2 weeks before the triple or quadruple therapy and for 4 weeks after the end of the therapy; that is, 4 weeks before the new Hp assessment (Figure **1**). Side effect evaluation was scheduled

immediately after the end of the PPI plus antibiotic therapy. Patients were also asked to take note of possible side effects of the probiotic, communicating these to the responsible physician throughout the course of the administration.

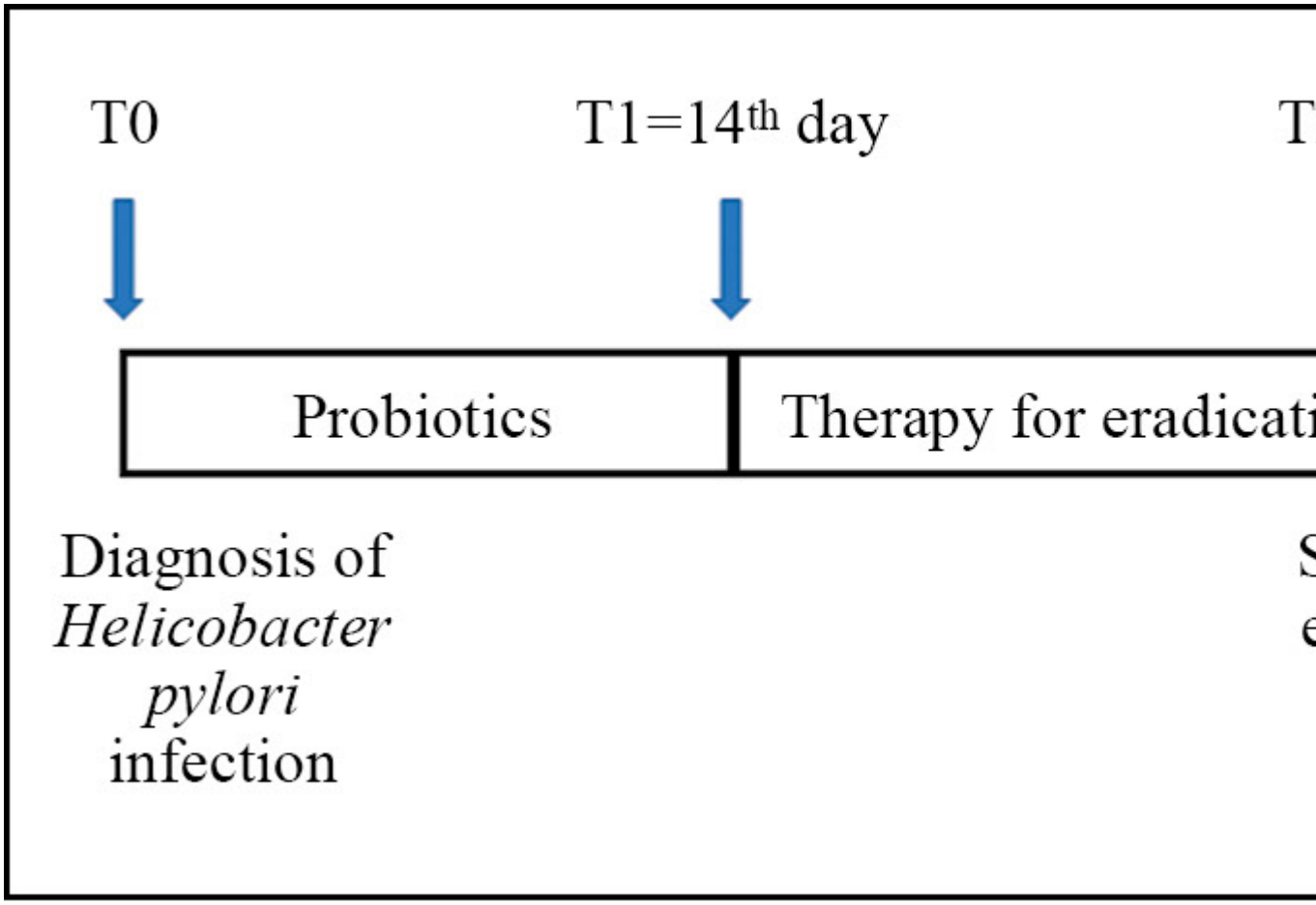

Figure 1.—Treatment scheme.

Group sizes

Our retrospective study compared 4 treatment groups: A) 10-day sequential therapy; B) 10-day sequential therapy plus probiotic; C) 10-day concomitant therapy; D) 10-day concomitant therapy plus probiotic. Due to the retrospective nature of our study, we have analyzed only those patients ending the study with a declared adherence to therapy of not less than 95%. We have therefore evaluated the eradication rate according to a “per protocol analysis” and not according to an “intention-to-treat analysis”. Completing the entire therapy regimen were, respectively, 38, 35, 42, and 46 subjects. Seven patients from Groups A (6) and B (1) who demonstrated unsuccessful eradication were later inserted into Group D.

Analyzed parameters

As previously mentioned, the successful eradication of Hp was defined by the absence of fecal antigen or by a negative ^{13}C-urea breath test. Drug tolerability (0–10) and side effects in all groups were recorded at the end of the triple or quadruple therapy. At the end of the study, the microbiota of some patients underwent analysis at MyMicrobiota, Pontenure, Piacenza, Italy. The protocols for sample collection, bacterial DNA extraction, 16S rRNA gene PCR amplification, sequencing, and the relevant database adopted by the laboratory are reported elsewhere.[18-21] For the identification of the observed operational taxonomic unit (OTU), an identity threshold of 100% for the amplicon genetic sequence was used.

Product

The probiotic product used in our study corresponds to iNatal Duo® sachets. Each sachet contains not less than 5 billion colony forming units (CFU) of *Enterococcus faecium* L3 (LMG P-27496) and 2 billion CFU of *Bifidobacterium animalis lactis* BB-12 (DSM 15954). The product is manufactured by Procemsa (Nichelino, Turin, Italy) and traded by the Pharmextracta S.p.A. (Pontenure, Piacenza, Italy). The product was notified to the Italian Health Authorities in 2016 (March), with the notification number 84053. For the 10-day sequential therapy, mono-ingredient generic drugs were used. For the 10-day concomitant therapy, commonly known as quadruple, Pylera (Allergan Pharmaceuticals, Dublin, Ireland) was used.

Statistical analysis

The difference in terms of outcome was determined using the two-tailed Wilcoxon-Mann-Whitney test. The difference between serial percentages of stool bacteria within the same group was tested by the paired *t*-test. The statistical software used was JMP 10 for Mac OsX and the threshold for statistical significance was 95%.

Results

The demographic features of the analyzed patients and the side effects of the therapies are reported in Table **I**. As can be seen, the groups are overlapping in terms of the main characteristics. Very significant differences in the reported side effects are demonstrated within the two pairs of groups. Probiotic administration reduced the incidence of nearly all side effects reported. Among these, some reductions were highly significant, namely vomiting, constipation, diarrhea, and bad taste in the mouth. Proportionally, tolerability was also higher in the two groups where probiotics were administered as an adjuvant therapy. Eradication rates, as determined using a per protocol analysis, are shown in Table **II**. Sequential therapy was successful in 31 subjects out of 38; the add-on with probiotics rendered the therapy successful in 33 of 35 subjects, with a difference of approximately 13% between the two groups. Quadruple therapy resulted in eradication in 37 of 42 subjects; the add-on with probiotics led to an eradication rate of 45 of 46, with a difference of approximately 9% between the two groups. Table **III** shows the difference in terms of the gut microbiota. The analysis was performed on several subjects from each group and the analytical report appears to be too complex to be completely evaluated (the MyMicrobiota report describes several hundreds of taxa). We have thus evaluated in our retrospective analysis the α-biodiversity only, through rarefaction curves, which incorporate the total number of OTUs, along with some selected taxa (those modifiable by probiotic administration, either directly or through mechanisms of co-variance). As shown, the rarefaction curves appear to be lower, meaning less gut richness, in groups A and C, and significantly higher in groups B and D where probiotics were administered. All probiotic taxa, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Streptococcus* were significantly higher in the two groups supplemented with probiotics, excepting *Streptococcus* in Group B where just a tendency was observed. *Escherichia*, because of the existence of the strain *Escherichia coli* Nissle 1917, traded in Europe as Mutaflor®, could be considered a "probiotic."[22] Conversely, its presence in the gut microbiota, can have, especially with very high values, a negative meaning also. As can be seen, a significantly higher abundance of *Escherichia* was found in the groups where probiotics were not administered, possibly demonstrating a substantial worsening of the microbiota composition.

TABLE I.—*Demographic features (average±standard deviation) and reported side effects in the four study groups.*

Parameter	Group A (N.=38)	Group B (N.=35)	P value (A *vs.* B)	Group C (N.=42)	Group D (N.=46)	P value (C *vs.* D)
Age, years	51.2±11.4	53.4±10.6	NS	49.8±10.7	50.7±12.5	NS
Sex ratio, M/F	20/18	18/17	NS	20/22	22/24	NS
BMI, kg/m^2	24.6±4.3	25.2±3.9	NS	23.9±3.4	24.2±3.6	NS
Side effects*						
Nausea	4	2	NS	7	3	NS
Vomiting	6	1	<0.05	8	2	<0.05

Constipation	6	0	<0.01	9	1	<0.01
Diarrhea	7	1	<0.01	10	2	<0.01
Bad taste in mouth	8	2	<0.01	15	7	<0.01
Headache	3	2	NS	3	1	NS
Fatigue	3	2	NS	4	1	NS
Poor sleep quality	3	3	NS	5	2	NS
Total side effects	40	13	<0.05	61	19	<0.01
Tolerability	6.8±0.8	8.9±0.5	<0.05	5.2±2.0	8.1±1.4	<0.01

BMI: Body Mass Index; Group A: sequential therapy only; Group B: sequential therapy plus probiotic; Group C: quadruple therapy; Group D: quadruple therapy plus probiotic; NS: not significant.
*Number of subjects having reported a side effect for at least one day.

TABLE II.—*Eradication rates for Helicobacter pylori infection in the four study groups.*

Parameter	Group A (N.=38)	Group B (N.=35)	P value (A *vs.* B)	Group C (N.=42)	Group D (N.=46)	P value (C *vs.* D)
N. of subjects	31	33	<0.05	37	45	<0.05
Percentage of subjects	81.6%	94.3%		88.1%	97.8%	

Group A: sequential therapy only; Group B: sequential therapy plus probiotic; Group C: quadruple therapy; Group D: quadruple therapy plus probiotic.

TABLE III.—*Evaluation of rarefaction curves and probiotic taxa in fecal samples from some of the patients from the four groups at the end of treatment.*

Microbiota parameters	Group A (N.=10)	Group B (N.=12)	P value (A *vs.* B)	Group C (N.=11)	Group D (N=13)	P value (C *vs.* D)
Biodiversity (N. of OTUs)	110±35	132±29	<0.05	102±39	138±31	<0.01
Bifidobacterium, %	0.00114	0.96383	<0.001	0.00036	0.82965	<0.001
Enterococcus, %	Undetected	0.02342	<0.001	Undetected	0.03298	<0.001
Lactobacillus, %	0.00042	0.04356	<0.001	Undetected	0.03987	<0.001
Lactococcus, %	0.00523	0.02343	<0.001	0.00087	0.01546	<0.001
Streptococcus, %	0.38872	0.42356	NS	0.13221	0.29342	<0.05
Escherichia, %	2.34431	1.00983	<0.05	4.12435	1.19763	<0.01

Group A: sequential therapy only; Group B: sequential therapy plus probiotic; Group C: quadruple therapy; Group D: quadruple therapy plus probiotic; OTU: operational taxonomic unit.

Discussion

This study is the retrospective analysis of the results that we, through our routine practice, have obtained in Hp treatment between June 2017 and May 2019 by adding, to PPI and antibiotic therapy, a probiotic product containing two strains, *Bifidobacterium animalis lactis* BB-12 and *Enterococcus faecium* L3. These have been described to play a role, when co-administered with a PPI and antibiotics, in reducing the clinical detection of Hp and in decreasing side effects also. According to our results, the probiotic adjuvant therapy indeed significantly reduced the occurrence of side effects by more than 60%, and significantly improved eradication rates by approximately 13% *versus* sequential therapy, and by approximately 9% *versus* quadruple therapy. Such a difference is likely due to the greater efficacy of the 10-day concomitant regime *versus* the 10-day sequential one. It is worth noting that seven patients previously treated with sequential therapy (six subjects, within Group A) and with sequential therapy plus probiotic (one subject, within Group B), who did not demonstrate successful Hp eradication, were re-enrolled in the study with all seven

subjects incorporated in the quadruple therapy plus probiotic arm (Group D). Following this, Hp eradication was seen in all seven subjects (data not shown in detail; that is, separate from Table **II**). Treatment with the probiotic product also seemed to improve the composition of the gut consortium. Despite the microbiota data being derived from only 10-13 subjects per group, largely because of the high cost of the analysis, the results clearly show a difference in terms of the microbiota composition between the groups treated with antibiotics and those also treated with colonizing probiotics. The rarefaction curves, higher in the probiotic groups, can in fact be interpreted as superior gut richness, a condition described as gut wellness. Moreover, all of the probiotic taxa, the two administered with the product (*Bifidobacterium* and *Enterococcus*) and the three not administered, which were also probably higher due to co-variance effects, had a significantly greater representation; this condition is also commonly described as gut wellness. Conversely, *Escherichia* taxa, often described as being inflammatory taxa within the gut microbial consortium, were more abundant in the two groups not administered probiotics as an add-on therapy. We believe that probiotic administration is responsible for the beneficial effects described here (fewer side effects, better eradication rates, improvement of the microbiota composition) through multiple mechanisms. Strain L3 is capable of a direct antagonistic effect against Hp by the release of different bacteriocins that cause membrane stress in pathogens and that induce pore formation, leading to the loss of cytoplasm from the cell and the entry of water into the cell, resulting in bacterial lysis.[23] Strain L3 has also been reported to increase the gut content of *Lactobacillus* and *Bifidobacterium*, ensuring a better gut microbiota and a reduced likelihood of diarrhea.[24] Strain BB-12 has been widely reported to increase the total count of gut *Bifidobacterium*, thereby improving the whole gut consortium.[25] BB-12 has also been described as reducing the gut presence of bacteria known to be strong producers of H_2, which Hp is known to use for nourishment.[14, 26]

Limitations of the study

We are aware that our retrospective study has limitations that could have influenced our conclusions. First of all, this has not been a prospective, randomized, double-blind, placebo-controlled clinical trial where the findings would be of greater significance. Second, our observations have been carried out on only 161 patients, divided into four groups, where seven patients were counted twice. However, we do not think that our trial scheme, not using probiotics during the antibiotic regimen, limits the validity of our results. As for any other bacterial strain used as a probiotic, both strain BB-12 and strain L3 are highly sensitive to the antibiotics used.[27, 28]

Conclusions

However, despite all these, and perhaps more limitations, we believe that our study allows us to confirm the general efficacy of probiotics both in reducing side effects due to the therapies commonly used to eradicate Hp and in increasing the eradication rate of Hp. Of course, this is particularly applicable to the two strains (BB-12 and L3) administered here. Moreover, even if a larger number of patients is surely needed, our findings also highlight a possible positive effect exerted by these two strains on gut microbiota composition. In conclusion, add-on therapy with probiotics, at least in our experience, is possibly a useful medical tool in the eradication of Hp.

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Conflicts of interest.—Francesco Di Pierro is part of the scientific board of Pharmextracta. All other authors have no conflicts of interest.

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