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ORIGINAL ARTICLE

Clinical efficacy of *Lactobacillus reuteri*-containing lozenges in the supportive therapy of generalized periodontitis stage III and IV, grade C: 1-year results of a double-blind randomized placebo-controlled pilot study

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Abstract

Objective The aim of this 12-month mono-centre double-blind randomized placebo-controlled clinical study was to evaluate the efficacy of *Lactobacillus reuteri*-containing lozenges during the supportive therapy of generalized periodontitis stage III and IV, grade C (GPIII-IVC) patients.

Material and methods Twenty treated GPIII-IVC patients were randomly divided into 2 groups. The test group received two 3-month-long administrations of *L. reuteri* (2 lozenges/day after brushing) with a 3-month washout period, while the control one received a placebo. Outcome measures were tooth survival, complications and adverse events, change in probing pockets depth (PPD), change in probing attachment level (PAL), presence of bleeding on probing (BOP) and patient's evaluation of treatment. Measurements were collected at 3, 6, 9 and 12 months.

Results At 1 year, no dropout, tooth loss, complications or adverse event were recorded. Mean PPD and mean PAL and percentages of sites with BOP were statistically improved ($p < 0.05$) compared with baseline in both groups, while more PPD reduction at all time points ($p < 0.05$) and more PAL gain at 6 months and more BOP reduction at 6 and 9 months were found in the probiotic group ($p < 0.05$).

Conclusions Within the limitation of the study, the use of *L. reuteri* probiotics lozenges improved some clinical outcomes in treated GPIII-IVC patients during maintenance therapy. Studies with a larger number of patients are needed to confirm these data.

Clinical relevance The use of *L. reuteri* probiotic lozenges could be considered as an adjunct in the maintenance therapy of GPIII-IVC patients.

Keywords Periodontitis · Probiotics · *Lactobacillus reuteri* · Clinical efficacy

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Introduction

Periodontal diseases are inflammatory driven pathologies caused by the immune response to the bacterial challenge. While traditional treatment concepts address the reduction of the bacterial load, among the new therapeutic approaches, the modification of the biofilm gained attention [1]. Pathogens colonization can occur quickly after treatment [2] and can lead to the recurrence of the disease [3]. In this perspective, probiotics were recently introduced in the periodontal treatment [4].

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Probiotics are defined by the World Health Organization, and the Food and Agriculture Organization of the USA as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' [5].

The influence of probiotics on pathogens flora can derive from three principal modes of action: innate and acquired host defence modulation, production of antibacterial substances and competitive exclusion mechanism, i.e. increasing the number of beneficial bacteria and retarding/avoiding recolonization with pathological ones [4, 6].

In particular, *Lactobacillus reuteri* has been studied for its antibacterial and anti-inflammatory properties and it is the most used probiotic in the studies addressing CP treatment. It is a heterofermentative bacterium and the distinct strains have different characteristics [7]. In particular, two strains of *L. reuteri*, DSM17938 and ATCC PTA 5289 strains, showed a synergistic effect. *L. reuteri* strain DSM 17938 produces reuterin that acts as an antibiotic and induces oxidative stress on pathogens and it is resistant to proteolytic and lipolytic enzymes [8, 9]. *L. reuteri* strain ATCC PTA 5289 presented anti-inflammatory properties. It can inhibit inflammatory mediators suppressing TNF production from monocytoid cells [8] and interleukin-8 and interleukin-1 beta [10]. Pro-inflammatory cytokines IL-1β, TNFα and IL-8 were reduced in gingival crevicular fluid of patients with gingivitis [11] using *L. reuteri* compared with the placebo group.

L. reuteri can be found commonly in saliva and on rare occasion in subgingival samples, and this last issue suggested its potential use as a probiotic in periodontal patients [11].

The effects of probiotics on periodontitis have first been analysed in periodontitis induced dog model [3]. Two recent reviews [12, 13] addressed the clinical effect of probiotics as an adjunct therapy to SRP with respect to SRP alone or plus placebo in chronic periodontitis patients. Four studies with 130 patients [9, 11, 14, 15] were included in the review of Martin-Cabezas [12]. The results showed a statistically significant PPD reduction in moderate (− 0.18 mm, $p = 0.001$) and deep pockets (− 0.67 mm, $p < 0.001$), CAL gain (− 0.42 mm, $p = 0.002$) and BOP reduction (− 14.66%, $p = 0.003$) at 3 months. All the studies used the same probiotic, *L. reuteri*.

Three more studies [16–18] were considered by Ikram et al. [13]. The meta-analysis concluded that PPD reduction was debatable but CAL improved.

Both reviews pointed to potential effectiveness of *L. reuteri* as an adjunct to SRP in initial treatment of chronic periodontitis patients but underlined the limits of their conclusions due to the heterogeneity of the studies and to the few patients included. There is thus a need for more long-term randomized controlled studies. In particular, no study addressed the use of this probiotic during the supportive therapy and in particular in patients with severe forms of periodontitis. While the 1999 Classification World Workshop of the American Academy of Periodontology recognized Aggressive Periodontitis and Chronic Periodontitis [19], the 2017 Classification World Workshop describes periodontitis by staging and grading [20]. The new classification allows patients' case definition at presentation and it is based on the severity and extent of the disease, the complexity of management as well as the risk of progression. Patients meeting the criteria of periodontitis stage III and IV, grade C are considered to be affected by severe and advanced forms of periodontitis with a rapid rate of progression. If more than 30% of the teeth are involved, then periodontitis is considered generalized. This group of patients could particularly benefit from adjunctive means aiding in the maintenance of periodontal health.

The aim of this randomized controlled study was thus to evaluate the efficacy of *Lactobacillus reuteri* probiotics as an adjunct to Full Mouth Guided Biofilm Therapy (FM-GBT) during the supportive therapy of patients affected by generalized periodontitis stage III and IV, grade C (GPIII-IVC) over a 1-year follow-up.

The test hypothesis was that there was no difference in clinical outcomes when using or not the probiotic as an adjunctive mean to FM-GBT, against the alternative hypothesis of a difference.

The trial was reported according to the CONSORT statement for improving the quality of reports of parallel-group randomized trials (http://www.consort-statement.org/).

Material and methods

The study was a mono-centre, pragmatic, randomized clinical trial (RCT) of parallel-group design. The trial had 1-year duration. The principles outlined in the Declaration of Helsinki on clinical research involving human subjects were adhered to and the study was approved by the Ethical Committee of the University of Brescia (protocol n 0059683 NP 1473). The study was registered at the Register of the Hospital of Brescia (Spedali civili) (NP 1473—Study AgP2013).

Participants

Patients treated for generalized periodontitis stage III and IV, grade C (GPIII-IVC) at the Department of Surgical Specialties, Radiological Science and Public Health, School of Dentistry, Section of Periodontics, Brescia, Italy, between January 2017 and August 2017, were considered eligible for the study. They belong to a group which had diagnosis of aggressive periodontitis based on presence of CAL ≥ 5 mm and radiographic bone loss of ≥ 30% of root length affecting at least three permanent teeth other than first molars and incisors. Familial aggregation

was present [19]. According to the 2017 World Workshop classification, they all were affected by periodontitis stage III (severe) and stage IV (advanced) and grade C (% bone loss at worst tooth/age > 1.0; destruction exceeded expectation given biofilm deposit) [20]. The baseline examination was done 3 months after the active treatment was ended. No patients received surgical treatment.

Inclusion criteria were:

- patients with treated GPIII-IVC otherwise healthy: patients were considered treated when they had no pus, BOP < 25%, PI < 25% and less than 9% of PPD > 6 mm. [21]

Exclusion criteria were:

- pregnancy
- use of other probiotics supplements
- use of antibiotic in the previous 3 months
- history of adverse reactions to lactose or fermented milk products
- impossibility to come to the recall appointments
- not willing to follow the agreed protocol

If the patient had to use antibiotics, she/he had to discontinue the study. Intention to treat analysis would be applied to her/his data.

A written informed consent was obtained from each patient included after explanation of the risks and benefits of participating in this study. No change in the trial design was made after approval of the Ethics Committee (protocol no. 0059683 NP 1473).

Intervention

For each participant, age, sex and smoking habits were recorded. Anamnesis was updated. Baseline examination at 6 sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) recorded: plaque (PI), as present or absent after the use of a disclosing plaque agent (MIRA-2-TON®, 60 ml bottle, Hager Werken); bleeding on probing (BOP) as present or absent; probing pocket depth (PPD) and probing attachment level (PAL) in millimetre. Furcation involvement was indicated as no involvement or furcation involvement grade I, II and III accordingly to Lindhe Jan (''Textbook of Clinical Periodontology'' [22]).

A PCP-UNC 15 periodontal probe[1] was used with a gentle probing force by the same experienced hygienist (G P). Measurements were rounded to the nearest millimetre.

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Intra-examiner reproducibility was calibrated measuring PPD and PAL in one quadrant with at least 6 teeth on 5 patients. Measurements were repeated after 2 h and calibration was accepted since the measurements were consistent (in mm) in ≥ 90% of the cases.

After baseline recording, a session of Full Mouth Guided Biofilm Therapy (FM-GBT) was performed [23]. In brief, rubber protection was placed (OptraGate® Ivoclar Vivadent, Schaan, Liechtenstein) and a disclosing plaque agent (MIRA-2-TON®, 60-ml bottle, Hager Werken) was applied and rinsed. Stains and supragingival biofilm were removed with an erythritol powder (Plus®, EMS, Nyon, Switzerland) air-polishing device (Air Flow Master Piezon® EMS Nyon, Switzerland). Subgingival biofilm was removed with the same air-polishing device where pockets were ≤ 4 mm and with a dedicated point (Perioflow®, EMS, Nyon, Switzerland) where pockets were > 4 mm.

Calculus was removed with a piezoceramic device (Air Flow Master Piezon® EMS Nyon, Switzerland) with a slim tip (PS® EMS, Nyon, Switzerland).

The patients were then randomly divided into two groups:

Test group: FM-GBT + probiotics lozenges (Reuterin OS ®, BioGaia, Stockholm, Sweden) (2 times a day for 12 weeks).
Control group: FM-GBT + placebo lozenges (2 times a day for 12 weeks).

The lozenges had the same look. The active ones contained the association of *L. reuteri* DSM 17938 and PTA 5389.

Reinforcement of oral hygiene instruction was given at every visit if needed. The disclosing plaque agent was used as a guide for showing dedicated oral hygiene instruments to the patients.

Each patient was given a box with the exact number of tablets to be taken for 3 months and the lozenges had exactly the same aspect and flavour in the test and control groups. The patient was instructed to suck one lozenge in the morning and one at night, after tooth brushing, and was instructed not to use any other probiotic tablets during the course of the study.

The boxes were returned to check the compliance in the third month. A patient was considered compliant if less than 20 lozenges were not taken during 3-months course.

Follow-up visits were scheduled every 3 months; examination, eventual reinforcement of oral hygiene instruction and FM-GBT were provided.

After 3 months of therapy with probiotics, a 3-month washout period followed. After the 6-month control visit, another course of 3 months of probiotics was provided,

followed by a 3 months free one (Fig. 1). Measurements were taken at 3, 6, 9 and 12 months.

The primary outcome measure was:

Change in mean probing pocket depth (PPD): change in mean value in mm for each patient was calculated.

Secondary outcome measures were:

Tooth survival: the reason for tooth loss was recorded.
Complications and adverse events: any complication and adverse events were recorded and reported by the blinded assessors.
Change in the percentage of sites with PPD > 4mm: change in percentage of sites with PPD > 4mm for each patient was calculated.
Change in mean probing attachment level (PAL): change in the mean value for each patient was calculated.
Change in bleeding on probing (BOP): change in % of sites with BOP for each patient was calculated.
Patient's evaluation of treatment: at the end of the first course and of the second course of probiotic administration (3 and 9 months), patients were asked to complete an anonymous questionnaire regarding their satisfaction with the treatment, in the absence of the treating clinician.

1) Do you think that probiotic supplement improved your mouth health?
Possible answers: 0 No 1 Average 2 Yes

2) Was it difficult to follow the protocol assigned?
Possible answers: 0 No 1 Average 2 Yes

3) Would you advise this type of treatment to friends with the same problem?
Possible answers: 0 No 1 Average 2 Yes

4) Would you have the same treatment again?
Possible answers: 0 No 1 Average 2 Yes

Measurements were taken by a blinded measurer (GG) at baseline, 3, 6, 9 and 12 months.

A block randomization list was made by a computerized programme following the Wichmann-Hill model. The randomization code was kept by the Company (Noos srl) providing the probiotic and the placebo boxes. The boxes were sent in identically numbered packages. The code was broken after the data were analysed.

Statistical analysis

Sample size was computed assuming a repeated measurement ANOVA design for testing a within-between subject interaction (time and treatment) effect size of 0.8 according to Cohen [24] four within-subject measurement, two groups and all other parameters set to defaults. Considering a power of 80% and a significance level of 5%, we estimated a sample size of 10 patients per group (total sample size $N = 20$). Calculations were performed using G*Power (version 3.1.9.3) [25].

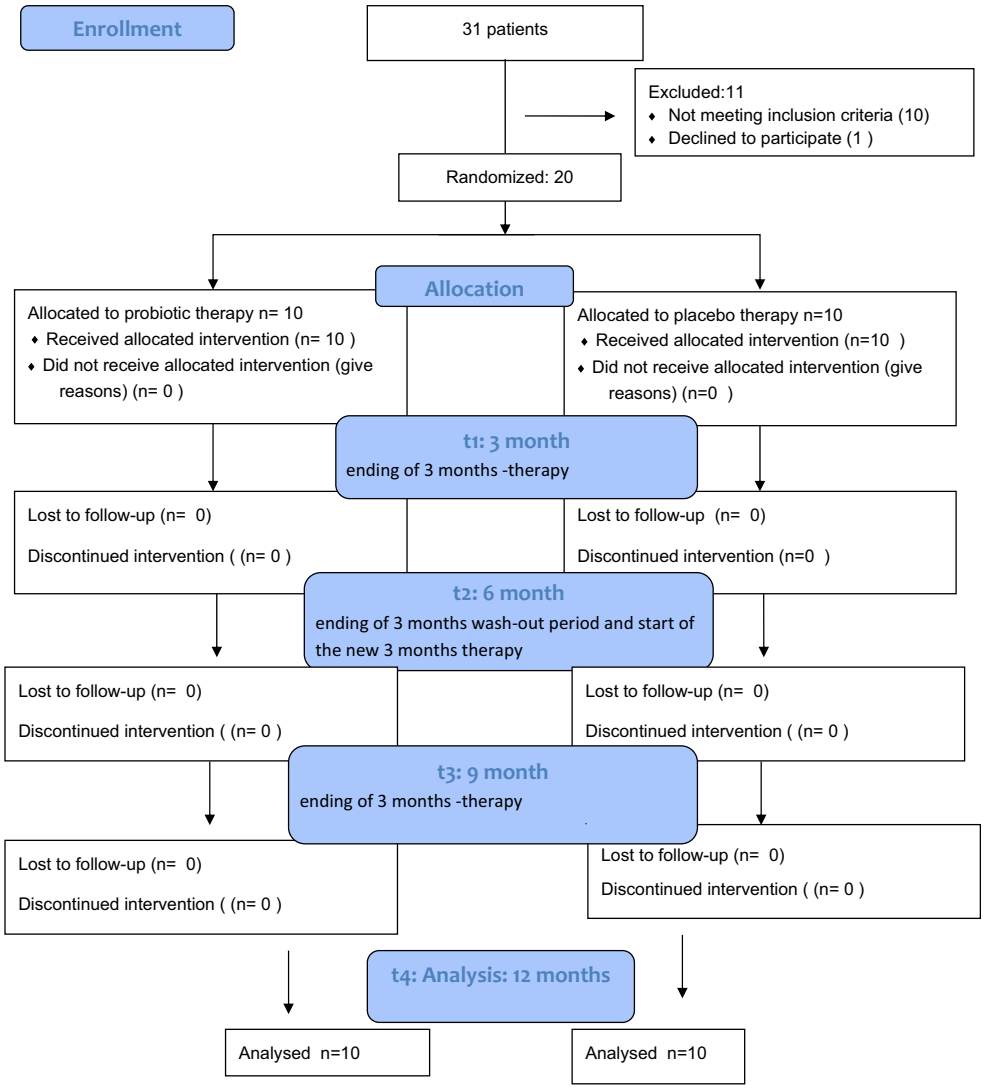
All data analyses were carried out according to a pre-established analysis plan by a biostatistician (S.C.) with expertise in dentistry and blinded to group allocation. Being a randomized study, baseline adjustment for all outcomes considered was performed using a constrained estimation. Briefly, if any difference at baseline is present, assuming randomization was performed correctly, it must be a random event. It is therefore common practice to estimate longitudinal effects assuming an identical baseline value for all treatment groups [26].

All outcome measurements were modelled using mixed models (MM) using the site as unit of analysis. The usage of a MM allows to account for the hierarchical data structure, that is the correlation among measurements at site level within patient. Due to a substantial skewness in data distribution, both PPD and PAL were modelled using a generalized linear mixed model assuming a gamma distribution for residuals and identity link function.

Data are presented at baseline, 3, 6, 9 and 12 months as mean values for continuous outcomes (PPD and PAL) and percentages for binary outcomes (BOP and pathological sites). For continuous outcomes effect estimates are different, while for binary, outcomes odds ratios (OR) are reported.

All statistical analysis was performed using R (version 3.5.1) assuming a 5% level of significance.

Fig. 1 Flow diagram



Results

Thirty-one patients were screened for inclusion between October 2016 and February 2017. Twenty (8 males, 12 females between 31 and 70 years of age) patients were included in the study (Fig. 1). Reasons for exclusions were pregnancy (1 patient), not able to come to recall appointments (5 patient), not willing to join the study (1 patient) and presence of teeth with mobility III (4 patients). One patient was a smoker in the placebo group, while 3 were smokers in the probiotic one.

Baseline clinical characteristics are summarized in Table 1 and were similar in the two groups.

At 1-year follow-up, no change in health status, antibiotic use, drop out, to
oth loss and complications were recorded. During the first course of the therapy, mucosa burning for 3 days was described by one patient of the placebo group, while pigmentations were reported by one patient in the probiotic group. No adverse events were recorded in the second course of the therapy. One patient in the placebo group stated improvement in

Table 1 Baseline characteristics

	Baseline		
	Placebo	Probiotic	*p* value
PPD (mm mean (sd))	2.2 (1.31)	2.3 (1.22)	0.56
PAL (mm mean (sd))	3.2 (2.11)	3 (1.79)	0.83
BOP (%)	16.2	23.6	0.28
PPD > 4 mm (%)	6.0	5.2	0.69
Plaque (%)	18.6	22.4	0.59
Age (years mean (sd))	49.2 (8.93)	50.1 (10.85)	0.84
Male (%)	50	30	0.65
Smoker (%)	10	30	0.58
Mean teeth number (sd)	24.0 (4.06)	24.6 (4.70)	0.76

halitosis, while in the probiotic group, less gingival bleeding (2 patients), better plaque control (1 patient), oral freshness (1 patient) and improvement in pain (1 patient) were reported.

All patients were compliant with the therapy with the exception of two subjects in the probiotic group that did not take the lozenges for 30 days, one during the first course and one during the second one.

Mean PPD was statistically significant reduced ($p < 0.05$) compared with baseline within each group at all time points. The change in mean PPD favoured the probiotic group at all time points (Table 2).

Mean % of the site with PPD > 4 mm was significantly lower compared with baseline in probiotic group at 6, 9 and 12 months ($p < 0.001$) and in the placebo one at 9 and 12 months ($p < 0.05$). No statistically significant difference was found in % of sites with PPD > 4 mm between the groups (Table 2).

Mean PAL improved at all time point in both groups ($p < 0.001$) and was statistically significantly better in the probiotic group only at 6 months ($p < 0.05$) (Table 3).

Bleeding on probing was statistically significantly different from baseline at all time points within each group ($p < 0.05$). Differences between groups in % of BOP were found at 6 and 9 months, favouring the probiotic group (Table 3).

A post hoc power analysis was computed via simulation with 100 repetitions, using a likelihood ratio test comparing models with and without time-treatment interaction, which is the main effect of interest in the study. As expected, for mean PPD and BOP, we achieved a high power (at least 90%), while for mean PAL, power was higher than 70% and for PPD > 4 mm power was lower than 33%.

After the second course of the therapy, 85% of the patients thought that probiotic supplements improved their oral health (80% after the first course), 100% found it easy to follow the protocol assigned (90% after the first course), 100% would recommend the treatment to friends with the same problem (100% after the first course) and 100% would be willing to have the same treatment again (95% after the first course).

Discussion

This double-blind, placebo-controlled pilot clinical trial evaluated the clinical effect of *L. reuteri*-containing lozenges as adjuvants during supportive therapy of patients affected by generalized periodontitis stage III and IV, grade C (GPIII-IVC). The probiotic group showed a steeper reduction in PPD at 3, 6, 9 and 12 months follow-ups, a higher PAL gain at 6 months and an increased BOP reduction at 6 and 9 months, compared with the placebo group. The percentage of sites with PPD > 4 mm improved only in the probiotic group, even if no statistical difference between groups was found.

Table 2 Mean (95% CI) probing pocket depth (PPD) and percentage of sites with PPD > 4 mm (95% CI) at baseline, 3, 6, 9 and 12 months: differences within a group related to time (time effect) and between groups at different time points (time-treatment)

		Placebo			Probiotic			Time-treatment	
PPD (mm)		Mean	Time effect	*p* value	Mean	Time effect	*p* value	Delta time	*p* value
	Baseline	2.23 (2.06, 2.40)	–	–	2.23 (2.06, 2.40)	–	–	–	–
	3 months	2.15 (1.97, 2.32)	− 0.09 (− 0.17, − 0.01)	< 0.05	2.05 (1.88, 2.23)	− 0.18 (− 0.26, − 0.10)	< 0.001	− 0.09 (− 0.18, − 0.01)	< 0.05
	6 months	2.15 (1.98, 2.33)	− 0.08 (− 0.16, 0.00)	0.073	1.96 (1.78, 2.13)	− 0.28 (− 0.36, − 0.19)	< 0.001	− 0.20 (− 0.28, − 0.11)	< 0.001
	9 months	2.12 (1.94, 2.29)	− 0.11 (− 0.20, − 0.03)	< 0.001	1.86 (1.69, 2.04)	− 0.37 (− 0.45, − 0.29)	< 0.001	− 0.26 (− 0.34, − 0.17)	< 0.001
	12 months	1.92 (1.75, 2.09)	− 0.31 (− 0.39, − 0.24)	< 0.001	1.76 (1.59, 1.93)	− 0.47 (− 0.55, − 0.40)	< 0.001	− 0.16 (− 0.24, − 0.08)	< 0.001
PPD > 4 mm		Mean (%)	Time effect (OR)	*p* value	Mean (%)	Time effect (OR)	*p* value	Ratio time (%)	*p* value
	Baseline	4.39 (2.49, 7.73)			4.39 (2.49, 7.73)				
	3 months	3.35 (1.74, 6.45)	0.76 (0.48, 1.21)	0.57	2.69 (1.36, 5.31)	0.61 (0.37, 1.01)	0.061	0.80 (0.49, 1.30)	0.37
	6 months	2.96 (1.53, 5.76)	0.68 (0.42, 1.09)	0.17	1.83 (0.89, 3.79)	0.42 (0.24, 0.74)	< 0.001	0.62 (0.37, 1.05)	0.07
	9 months	2.48 (1.26, 4.90)	0.57 (0.34, 0.93)	< 0.05	2.00 (0.98, 4.09)	0.46 (0.26, 0.79)	< 0.001	0.81 (0.48, 1.37)	0.42
	12 months	2.64 (1.34, 5.18)	0.60 (0.37, 0.98)	< 0.05	1.95 (0.95, 3.99)	0.44 (0.25, 0.77)	< 0.001	0.74 (0.43, 1.25)	0.25

Table 3 Mean (95% CI) probing attachment level (PAL (mm)) and percentage of sites with BOP (95% CI) at baseline, 3, 6, 9 and 12 months: differences within a group related to time (time effect) and between groups at different time points (time-treatment)

		Placebo			Probiotics			Time-Treatment	
PAL (mm)		Mean	Time effect	*p* value	Mean	Time effect	*p* value	Delta time	*p* value
	Baseline	3.24 (2.82, 3.65)	•	•	3.24 (2.82, 3.65)	•	•	•	•
	3 months	3.05 (2.63, 3.47)	− 0.18 (− 0.29, − 0.08)	< 0.001	3.06 (2.64, 3.48)	− 0.17 (− 0.27, − 0.07)	< 0.001	0.01 (− 0.10, 0.12)	0.84
	6 months	3.05 (2.63, 3.48)	− 0.18 (− 0.29, − 0.07)	< 0.001	2.95 (2.53, 3.37)	− 0.29 (− 0.39, − 0.19)	< 0.001	− 0.11 (− 0.21, − 0.00)	< 0.05
	9 months	2.92 (2.50, 3.34)	− 0.31(− 0.42, − 0.21)	< 0.001	2.83 (2.41, 3.25)	− 0.41 (− 0.50, − 0.31)	< 0.001	− 0.10 (− 0.20, 0.01)	0.06
	12 months	2.74 (2.32, 3.16)	− 0.50 (− 0.60, − 0.40)	< 0.001	2.74 (2.32, 3.16)	− 0.50 (− 0.59, − 0.41)	< 0.001	− 0.00 (− 0.10, 0.10)	0.94
BOP (%)		Mean (%)	Time effect (OR)	*p* value	Mean (%)	Time effect (OR)	*p* value	Ratio time (%)	*p* value
	Baseline	22.42 (15.72, 31.99)			22.42 (15.72, 31.99)				
	3 months	16.23 (10.76, 24.47)	0.72 (0.54, 0.97)	< 0.05	13.23 (8.84, 19.80)	0.59 (0.45, 0.77)	< 0.001	0.82 (0.62, 1.08)	0.15
	6 months	16.77 (11.13, 25.25)	0.75 (0.56, 1.00)	< 0.05	9.57 (6.32, 14.49)	0.43 (0.32, 0.57)	< 0.001	0.57 (0.43, 0.76)	< 0.001
	9 months	16.33 (10.83, 24.63)	0.73 (0.54, 0.97)	< 0.05	10.59 (7.02, 15.97)	0.47 (0.36, 0.62)	< 0.001	0.65 (0.49, 0.86)	< 0.05
	12 months	11.10 (7.23, 17.04)	0.49 (0.36, 0.68)	< 0.001	10.80 (7.16, 16.27)	0.48 (0.37, 0.63)	< 0.001	0.97 (0.72, 1.32)	0.85

The study analysed treated GPIII-IVC patients inserted in a maintenance programme. The patients belong to a group selected according to a protocol written in 2013 at the Brescia Hospital. As the 1999 World Workshop Classification removed any age-dependent differentiation criteria, the group initially received the diagnosis of generalized aggressive periodontitis based on the amount of attachment loss and bone destruction (CAL ≥ 5 mm and radiographic loss of 30% of root length affecting at least three permanent teeth other than first molar and incisors) that was not justified by the amount of microbial deposits and the age. The amount of destruction was not judged consistent with chronic periodontitis. Family aggregation was present. A new classification for periodontal diseases [20] was published after the completion of data collection and stated that there is little evidence that chronic and aggressive periodontitis are different diseases. Besides, the fast rate of progression belonging to Aggressive Periodontitis is not easy to assess at the initial examination. We found more appropriate to adopt the new classification system since the same inclusion criteria could be maintained and the patients' case description appeared more accurate. Using the new classification, all the patients could be classified as affected by periodontitis generalized stage III (severe) and IV (advanced) and grade C. Patients with less than 9% of PPD > 6 mm at the re-evaluation were considered treated since they could be considered at low risk of disease progression [21]. No patient lost teeth during the 1-year follow-up and this result is in accordance with a recent retrospective study [27] that showed a low risk for tooth loss in aggressive periodontitis patients inserted in a proper maintenance programme (0.075 teeth/per year). All patients showed a statistically significant improvement in clinical parameters between baseline and 1-year follow-up, underlining the importance of a correct supportive therapy. In the probiotic group, a small but statistically significant reduction in mean PPD was present at all follow-up appointments, while a higher CAL gain at 6 months, fewer sites with BOP at 6 and 9 months and a tendency for a smaller number of sites with PPD > 4 mm were observed. Such small differences can still be clinically significant for these patients characterized by a high risk of disease progression. The role of the probiotic could be different when acting during initial or during supportive therapy and its effect could be more pronounced working on a patient with a greater bacterial load. Nevertheless, in our study, the statistically significant greater reduction in mean PPD of 0.20 mm at 6 months, 0.26 mm at 9 months and 0.16 mm at 1 year was comparable with the difference of 0.18 mm at 3 months obtained in moderate PPD reported in a recent meta-analysis [12] in chronic periodontitis patients. Both studied groups showed a statistically significant reduction in sites with PPD > 4 mm at 9 and 12 months even if there was no statistically significant difference between groups. Only 4.39% of sites had PPD > 4 mm at baseline and this number was smaller at the 1-year follow-up (2.64% in the placebo and 1.95% in the probiotic group). The little number of residual deep pockets could mask the presence of a difference between groups

and studies with a higher number of patients and longer follow-ups are needed to confirm this hypothesis. In fact, one of the main limitations of this study is the little number of patients included. Even if a power calculation was performed a priori and the calculated sample size of 20 was reached, few residual pockets were present in this well-maintained group of patients. This can result in an increased probability of type II error and the study could be underpowered to detect differences between groups. The same issue was raised by Teughels et al. [14]: 63 patients per group instead of 10 were deemed necessary to provide 80% power with an alpha of 0.05 for change in PPD as indicated by a post hoc analysis based on the primary outcome measure. The few PPD > 4 mm present in both groups in our study can explain why the differences between groups were not significant, but the tendency showed more reduction in the probiotic group. The probiotic treated patients showed more than 50% reduction in presence of pathological pockets, while it was less than 40% in the placebo one. More than 10% difference can be clinically significant since it means that the probability of surgery or of disease progression was lessened in the probiotic group. Even small differences can be important in this group of patients. A statistically significant difference in percentage of sites, teeth and number of patients in need of surgery favouring the probiotic group was found by Teughels et al. [14] at 1-year follow-up.

Another limitation of the study is the 1-year follow-up. Periodontal patients are inserted in a lifelong maintenance programme and it would be useful to know the long-term effect of the use of probiotics.

To our knowledge, this is the first study that addressed the use of a probiotic in the maintenance of GPIII-IVC patients so comparison with previous studies can be difficult. Different types of probiotics could be used as an adjunct to periodontal therapy. A recent study [28] dealt with the use of heat-killed *Lactobacillus plantarum* L-137 (HK L-137) in chronic periodontitis patients undergoing supportive periodontal therapy. At 12 weeks, fewer sites with PPD ≥ 4 mm were found, while there was no difference in mean PPD change. In our study, the use of a different probiotic, severe periodontitis patients and PPD > 4 mm were analysed so no direct comparison can be made.

A few studies evaluated clinical and microbiological effects of *L. reuteri*-containing lozenges in non-treated chronic periodontitis patients as an adjunct to SRP [9, 11, 14, 15]. The modality and duration of the supplementation and the evaluation periods were different among the studies but their results suggested a positive effect of the probiotic. Follow-up periods varied from 3 weeks to 1 year and administration periods from 3 weeks to 3 months.

Patients in this study took the lozenges for 3 months following the indications of Teughels et al. [15] who addressed severe generalized adult periodontitis, a group of patients judged similar to the one included in our study. No study up to now compared the results obtained using different protocols of probiotic administration. The dosage and frequency that would yield the best clinical result is unclear and this is one of the limitations of the study. We do not know which results could be obtained with other modalities of administration. It would be interesting to know the shortest time in which probiotics can exert a favourable result so as to limit the problem of compliance that could be a bias in the study. Even if the number of lozenges used were monitored, we do not know if the patients every time really melt the lozenges slowly in the mouth or if they swallowed them since they did the procedures at home. A more controlled way of administration would be advisable.

In all the studies examined, the probiotic was administrated once and the probiotic group had a progressive improvement in clinical parameters reflecting disease at all time points up to 1 year [11, 15]. A 3-months interval between the probiotics administration was decided based on the microbiological findings of Tekce et al. [11]. After a 3-week administration, *L. reuteri* was present on day 90 but no longer after 6 months, even if the proportion of obligate anaerobes showed a statistically significant difference in favour of the probiotic group up to 180 days [11]. Since these bacteria seem not to be able to obtain a stable colonization of the mouth, in order to maximize the probiotic effect, a 3-month interval was deemed necessary to maintain *L. reuteri* presence in the subgingival sites, although colonization is not considered essential to obtain the benefits of probiotics, at least in the gastrointestinal tract [29].

The clinical benefit of treatment should be judged against its adverse effects and it should take into account the preferences of the patients [30]. Patient-reported outcomes (PROs) [31] were included in the protocol of this study and showed that taking the lozenges was well accepted by the patients. They had the perception that their oral health improved and that the protocol was easy to follow. They were willing to do the same therapy again and to recommend the treatment to their friends with the same problem. The only one patient that during the first course was not willing to redo the treatment had reported pigmentation, but his opinion changed during the second course of therapy.

The achieved results should be compared with the results obtained by other adjunctive therapies. A recent meta-analysis compared different therapies adjunctive with scaling and root planning (SRP) in the treatment of chronic periodontitis patients [32]. Systemic antimicrobials, subantimicrobial dose of doxycycline used as a systemic host modulator, locally delivered antimicrobials (chlorhexidine chips, doxycycline hyclate gel, minocycline microspheres) and the use of nonsurgical lasers (photodynamic therapy with a diode laser, a diode laser, neodymium: yttrium-aluminium-garnet lasers and erbium lasers) were examined. Four adjunctive therapies were judged

beneficial at low/moderate level of evidence: systemic subantimicrobial-dose doxycycline, systemic antimicrobials, chlorhexidine chips and photodynamic therapy with a diode laser. They gave an average improvement in CAL in the range of 0.2–0.6 mm that is comparable with the improvement (0.42 mm) reported with the adjunctive use of probiotics in chronic periodontitis patients [12]. These results suggest that probiotics can be considered as an adjunct therapy at least in chronic periodontitis patients even if proper randomized controlled studies should be run to assess the efficacy of probiotics. A recent systematic review [33] reported a statistically significant CAL gain and PPD reduction using adjunctive systemic amoxicillin and metronidazole in aggressive periodontitis patients during active treatment, but there were no data on patients during maintenance. The potential use of probiotics instead of antibiotics in the treatment of aggressive periodontitis was proposed in one study [34]. Thirty patients were randomized to receive the probiotic alone (*L. brevis*), the probiotic in combination with doxycycline or doxycycline alone. No control group was present. Statistically significant reduction of PPD and gain in CAL was obtained with no differences among groups.

Conclusion

Within the limitation of this study, the adjunctive use of *L. reuteri* probiotic lozenges in a group of well-maintained generalized periodontitis stage III and IV, grade C patients showed a statistically significant improvement in mean PPD and partially in CAL and BOP during a 1-year follow-up. The use of probiotics could be considered as an adjunct during the maintenance therapy of generalized periodontitis stage III and IV, grade C patients, but more studies with a higher number of patients and longer follow-up are needed to confirm these results and to assess the ideal administration modality of the probiotic.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest. Author MGG has received a research grant from company Noos, who provided to the University free the probiotic used in the study. However, data belonged to the authors and by no means, the company interfered with the conduct of the study

Ethical approval All procedure involving humans have been approved by the institutional ethics committee of the University of Brescia (protocol n 0059683 NP 1473) and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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