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Clinical Effects of Locally Delivered *Lactobacillus reuteri* as Adjunctive Therapy in Patients with Periodontitis: A Split-Mouth Study

Irina-Georgeta Sufaru [1,*,†], Luminita Lazar [2,†], Dorina-Cerasella Sincar [3,†], Maria-Alexandra Martu [1,*,†], Liliana Pasarin [1,†], Elena-Odette Luca [4,†], Ada Stefanescu [5,†], Eliza-Maria Froicu [6,†] and Sorina-Mihaela Solomon [1,†]

1 Department of Periodontology, Grigore T. Popa University of Medicine and Pharmacy Iasi, Universitatii Street 16, 700115 Iasi, Romania; liliana.pasarin@umfiasi.ro (L.P.); sorina.solomon@umfiasi.ro (S.-M.S.)
2 Department of Periodontology, Faculty of Dental Medicine, George Emil Palade University of Medicine and Pharmacy Targu Mures, Ghe. Marinescu Street 38, 540139 Targu Mures, Romania; luminita.lazar@umfst.ro
3 Department of Periodontology, Dunarea de Jos University of Medicine and Pharmacy Galati, Domneasca Street 47, 800008 Galati, Romania; cerasella.sincar@ugal.ro
4 Department of Implantology, Mobile Restorations and Technology, Grigore T. Popa University of Medicine and Pharmacy Iasi, Universitatii Street 16, 700115 Iasi, Romania; elena-odette.luca@umfiasi.ro
5 Department of Dental Prosthetics, Dunarea de Jos University of Medicine and Pharmacy Galati, Domneasca Street 47, 800008 Galati, Romania; ada.stefanescu@ugal.ro
6 Department of Oncology, Grigore T. Popa University of Medicine and Pharmacy Iasi, Universitatii Street 16, 700115 Iasi, Romania; eliza-maria_d_froicu@d.umfiasi.ro
* Correspondence: ursarescu.irina@umfiasi.ro (I.-G.S.); maria-alexandra.martu@umfiasi.ro (M.-A.M.)
† These authors contributed equally to this work.

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Abstract: Different methods and products have been investigated as measures of adjunctive therapy to scaling and root planing (SRP). Probiotic use has gained interest for this particular application, especially *Lactobacillus* spp. This split-mouth interventional prospective study aimed to evaluate the clinical effects of *L. reuteri* DSM 17938 with local application in periodontal pockets of severe periodontitis patients. The study was conducted on 40 subjects with stage 3–4 periodontitis who, based on a split-mouth model, followed SRP and SRP + *L. reuteri* solution in five weekly sessions. Probing depth (PD), clinical attachment loss (CAL), and bleeding on probing (BOP) were assessed at baseline and at three months after probiotic treatment completion. Both SRP and SRP + *L. reuteri* generated significant improvements of all three clinical parameters, but the changes were significantly better for SRP + *L. reuteri* treated sites. Therefore, we can conclude that adjunctive therapy with *L. reuteri* DSM 17938 could represent an interesting treatment option, particularly for severe periodontitis cases.

Keywords: periodontitis; adjunctive therapy; probiotics; *Lactobacillus reuteri*

1. Introduction

Periodontal disease represents an infectious disease, extremely widespread worldwide [1]. The main etiological factor is given by the periodontopathogenic microorganisms, among which we mention *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, organized in the bacterial biofilm [2]. The human body will react to bacterial aggression by activating non-specific and specific immune systems, generating the inflammatory reaction [3]. Of course, this interaction may involve grafting or a series of local and/or systemic risk factors that may influence the quantity or quality of the supra- and subgingival bacterial plaque, or the immune response capacity of the host, or even both components [4]. Following this exacerbated inflammatory response, the first clinical

changes appear in the superficial periodontal tissues (gingival tissues), a pathological phenomenon included in the group of gingivitis. If left untreated, inflammatory and bacterial products can affect the supporting periodontal tissues, such as periodontal ligaments, cementum, or alveolar bone, with gingivitis evolving into periodontitis, a disease that can have as a negative endpoint teeth loss [5].

Etiological periodontal therapy addresses precisely these factors that lead to the onset and evolution of destructive periodontal phenomena. The gold standard in periodontal therapy remains scaling and root planing (SRP), a non-surgical debridement therapy that aims to disorganize the bacterial biofilm and create biologically acceptable surfaces that allow the formation of a new periodontal attachment and prevent bacterial adhesion [6]. Over time, various forms of adjunctive therapy have been associated with SRP in order to improve the results and response of the human body to standard therapy. These forms of treatment include topically or systemically administered antibiotic/antiseptic therapy, or photoactivation therapy, among many other methods [7,8].

Particular attention was paid to the use of probiotics as an additional means in periodontal etiological therapy. Probiotics are viable microorganisms that, when administered in adequate amounts, provide a health benefit to the human body. The use of probiotics has been shown to be effective in controlling intestinal diseases and appears to act by resisting colonization and/or modulating the immune system [9]. Regarding the use of probiotics for the additional therapy of various diseases of the oral cavity, the strains of *Lactobacillus*, *Streptococcus,* and *Bifidobacterium* are the most frequently investigated [10].

A number of mechanisms of action have been proposed for probiotics, phenomena which include: (a) Direct interactions within the oral bacterial plaque, disruption of plaque biofilm formation by competition for binding sites on host tissues and for nutrients; probiotic species are also thought to produce a number of antimicrobial compounds (organic acids, hydrogen peroxide, peptides, bacteriocins, and anti-adhesion molecules) [11]; (b) Modulation of both innate and adaptive immune function, with an alteration of cytokine production and subsequent effects on general immunity [11]. Moreover, some probiotic species may improve mucin production and barrier function, regulate host defense peptides, as well as promote angiogenesis and wound healing [12].

The most common bacterial strains used as probiotics include the *Lactobacillus* species, and the genus reuteri appears to generate the most significant results and is the strongest of all [13]. *L. reuteri* ATCC 55730 was originally isolated from breast milk and may be present in humans on the lining of the gastric body and antrum, duodenum, and ileum [14]. *L. reuteri* ATCC 55730 was found to exhibit a potentially transferable resistance trait for tetracycline and lincomycin. Therefore, it was replaced with *L. reuteri* DSM 17938, a strain without undesired resistance [15].

Given the presented benefits of probiotics, starting from the hypothesis that their local administration in periodontal pockets would generate beneficial effects, we conducted an interventional study aimed at evaluating periodontal clinical parameters before and after supplementing SRP with *L. reuteri* DSM 17938 applied in periodontal pockets in patients with stages 3–4 periodontitis.

2. Materials and Methods

The study was performed on a group of 40 patients. The criteria for inclusion in the study were patients with periodontitis stages 3–4, with at least 20 teeth present on the arches and the study excluded smoking patients, patients with systemic diseases that could interfere with periodontal status, patients who have followed by antibiotic/antiseptic therapy or periodontal therapy in the last 3 months prior to the start of the study, and pregnant or lactating patients.

The study methodology was explained to each patient and signed informed consent was obtained. The methodology of the study complied with the rules set out in the Helsinki Declaration and approved by the "Grigore T. Popa" UMPh Ethics Committee/30.07.2020.

Each patient underwent a rigorous clinical examination to determine the following periodontal parameters: probing depth (PD), clinical periodontal attachment loss (CAL), and bleeding on probing index (BOP). The measurements were performed by two experienced and calibrated examiners, with an agreement of 99.85%, using the periodontal probe (Williams Color-Coded Probe PQW, Hu Friedy Mfg. Co., LLC, Chicago, IL, USA).

In order to eliminate the inter-individual variability, a split-mouth design was used for the therapeutic approach. Full-mouth ultrasonic scaling (Woodpecker UDS-A-LED, Guilin Woodpecker Medical Instruments CO. LTD, Guangxi, China) and root planing (Gracey curettes, Hu Friedy Mfg. Co., LLC, Chicago, IL, USA) were performed in one session for each subject.

In addition, local application of *L. reuteri* to periodontal pockets of two quadrants per patient was performed. The *L. reuteri* treatment was randomly assigned to either the right or the left halves of the dentition. The applied solution comprised 10^8 living CFU *L. reuteri* strain DSM 17938 per 0.2ml (Protectis®, BioGaia, Stockholm, Sweden), with a mean volume of 0.96 ± 0.3ml per subject. For the application, each site was isolated with cotton rolls, gently dried and *L. reuteri* solution was instilled in the pocket with a blunt syringe. The area was kept isolated for 10 min. The procedure was performed in the same session with SRP and repeated at 7, 14, 21, and 28 days, respectively. Forty-four patients were initially enrolled in the study but 4 of them (9.09%) could not follow all the treatment sessions and were, therefore, excluded. The clinical measurements were reprised at 3 months after the treatment completion (4 months from baseline).

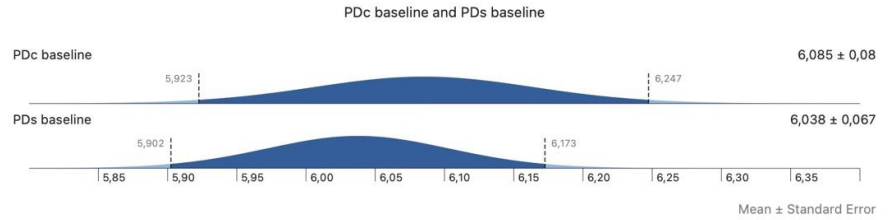
Oral hygiene instructions were given in terms of toothbrushing and dental flossing and the patients were required to avoid the use of antiseptics, antibiotics, anti-inflammatory drugs or any other probiotics.

All the data were registered for each subject and statistically analyzed (Microsoft Excel 2021 and Wizard 2 for Mac, Evan Miller®). A power analysis was performed for CAL as a parameter, estimating an 8% reduction, with a 90% power and alpha 0.05, generating a result of 32 subjects dimension group; we also estimated a 10% rate of abandon. A Shapiro–Wilk test was conducted as normality test. Normally distributed values were compared with the paired *t*-test and for non-normally distributed values we used the Mann–Whitney U test.

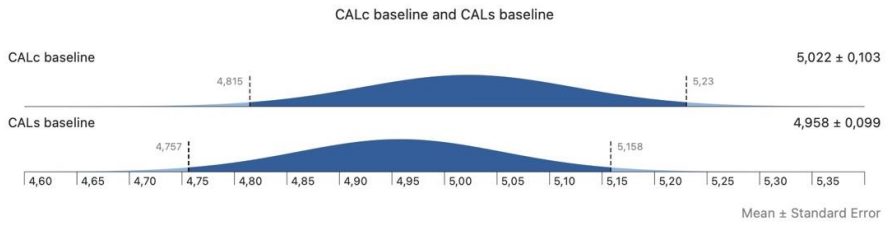
3. Results

Forty patients followed the present study, of which 19 subjects were male (47.5%) and 21 subjects were female (52.5%). The mean age was 48.65 ± 6.62 years old.

No significant differences were observed between study sites groups at baseline regarding probing depth, clinical attachment loss, or bleeding on probing ($p = 0.650$, $p = 0.650$ and $p = 0.595$, respectively) (Figure 1a–c).



(**a**)



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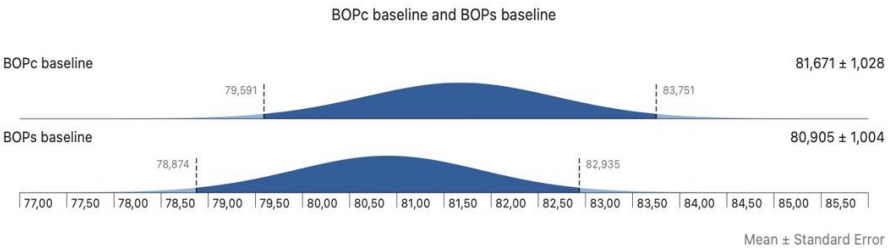


(**c**)

Figure 1. Comparaison at baseline between (**a**) probing depth values (PDc: probing depth in SRP alone sites; PDs: probing depth in SRP + *L. reuteri* sites); (**b**) clinical attachment loss (CALc: clinical attachment loss in SRP alone sites; CALs: clinical attachment loss in SRP + *L. reuteri* sites); (**c**) bleeding on probing values (expressed in %) (BOPc: bleeding on probing in SRP alone sites; BOPs: bleeding on probing in SRP + *L. reuteri* sites).

In the control group of sites who followed only SRP, we observed a significant reduction of all three examined parameters after three months (6.09 ± 0.51mm to 5.58 ± 0.49mm for PD; 5.02 ± 0.65mm to 4.65 ± 0.62mm for CAL; 81.67 ± 6.5 to 26.40 ± 9.54 for BOP) ($p < 0.001$ for all three parameters in the same study group) (Figure 2a–c).

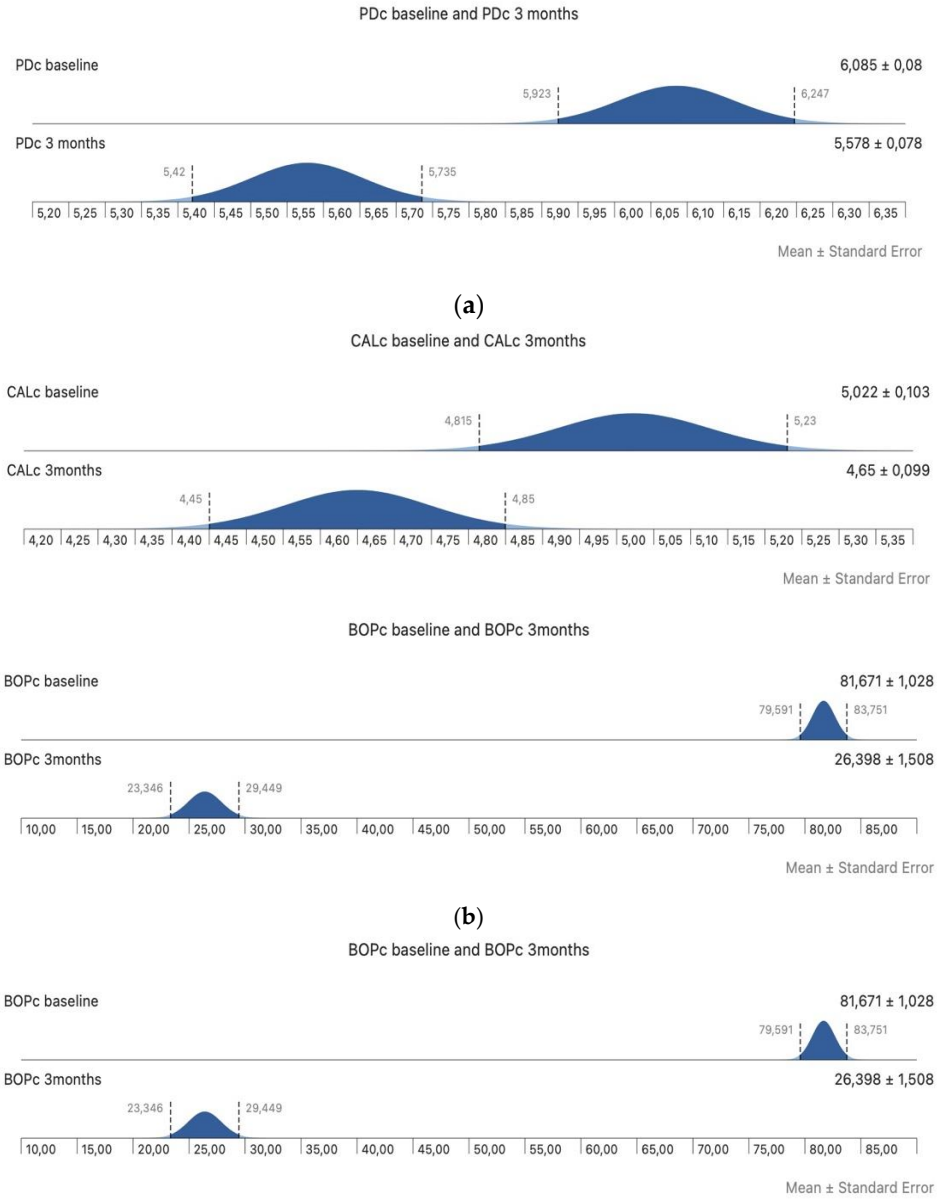
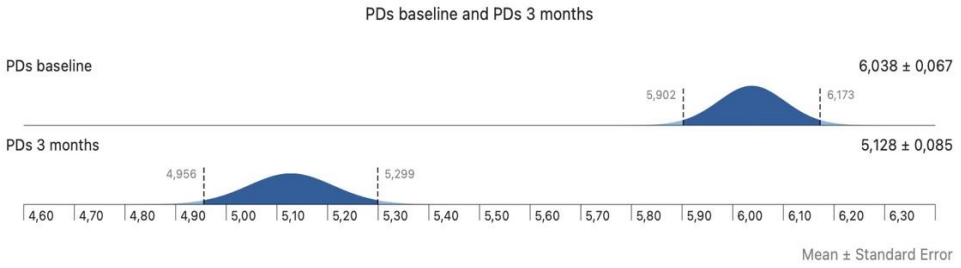
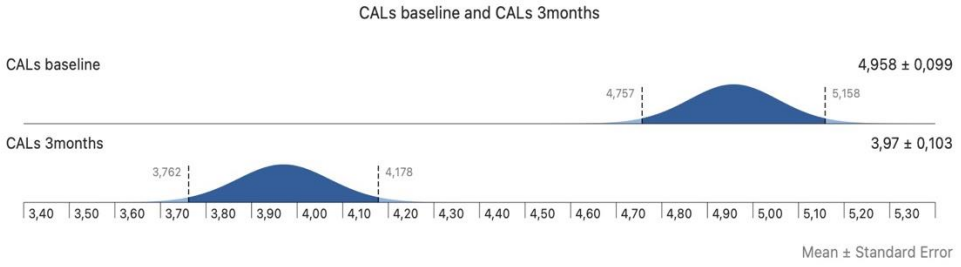


Figure 2. Comparaison at baseline and 3 months in control group between (**a**) probing depth values (PDc: probing depth in SRP alone sites); (**b**) clinical attachment loss (CALc: clinical attachment loss in SRP alone sites); (**c**) bleeding on probing values (expressed in %) (BOPc: bleeding on probing in SRP alone sites).

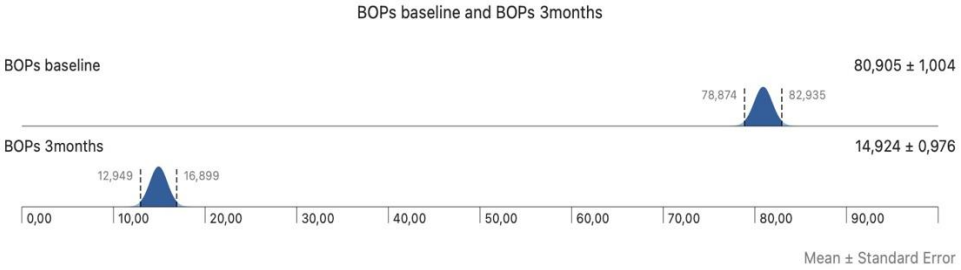
Significant differences were also observed for the study group who followed supplementary therapy with *L. reuteri* (reductions from 6.04 ± 0.42mm to 5.13 ± 0.54mm for PD; from 4.96 ± 0.63mm to 3.97 ± 0.65mm for CAL; from 80.90 ± 6.35 to 14.92 ± 6.17 for BOP; $p < 0.001$ for all three parameters) (Figure 3a–c).



(**a**)



(**b**)



(**c**)

Figure 3. Comparaison at baseline and 3 months in study group between (**a**) probing depth values (PDc: probing depth in SRP + *L. reuteri* sites); (**b**) clinical attachment loss (CALc: clinical attachment loss in SRP + *L. reuteri* sites); (**c**) bleeding on probing values (expressed in %) (BOPc: bleeding on probing in SRP + *L. reuteri* sites).

Moreover, the observed reductions in all three parameters were more significant for the subjects who also followed local therapy with *L. reuteri* in periodontal pockets ($p < 0.001$ for all three parameters) (Figure 4a–c).

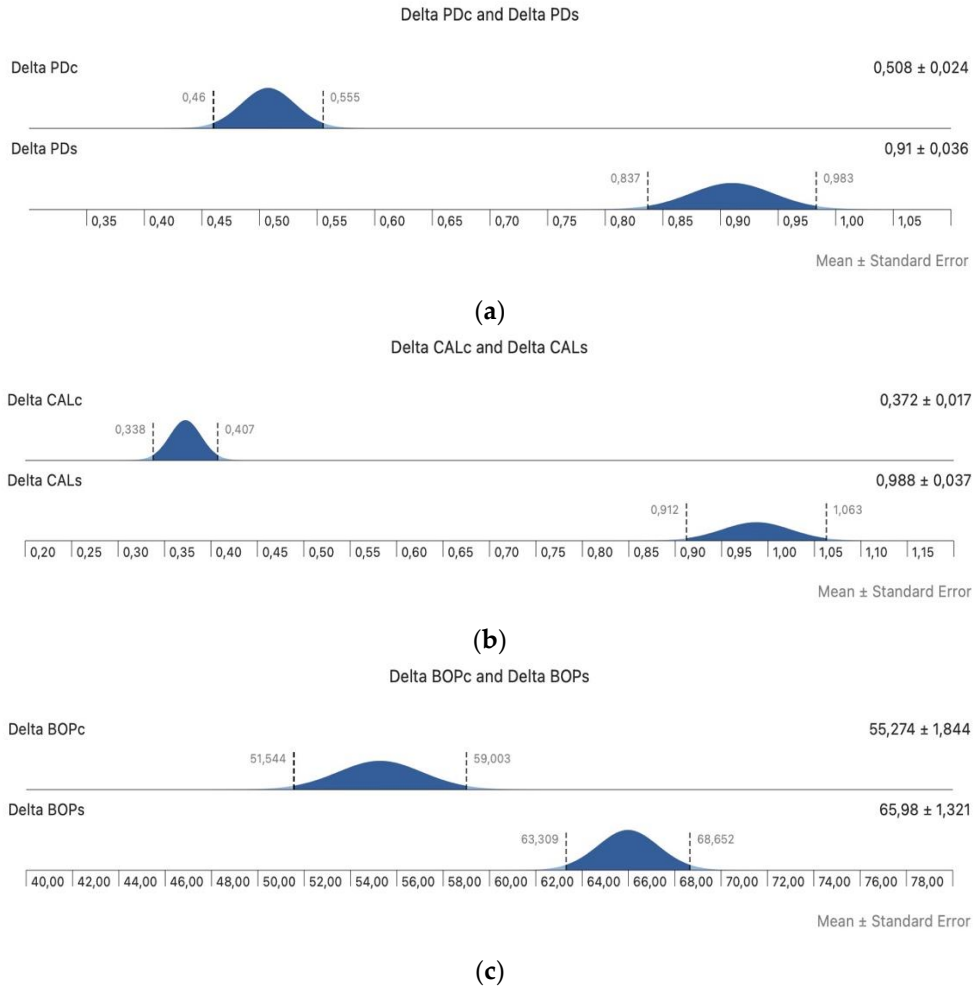


Figure 4. Comparaison between (**a**) difference in probing depth at 3 months and baseline for control group (Delta PDc) and study group (Delta PDs); (**b**) difference in clinical attachment loss at 3 months and baseline for control group (Delta CALc) and study group (Delta CALs); (**c**) difference in bleeding on probing at 3 months and baseline for control group (Delta BOPc) and study group (Delta BOPs).

4. Discussion

This interventional split-mouth prospective study aimed to evaluate the potential benefits of using instillations with *L. reuteri* in periodontal pockets in patients with severe periodontitis. Our results demonstrated that both SRP alone and SRP supplemented with *L. reuteri* treatment generated favorable results in terms of clinical parameters (probing depth, clinical attachment loss, bleeding on probing index) at three months after *L. reuteri* treatment completion. More important, the attachment gain and the inflammation resolution (measured by BOP) were more significative for those sites that were subjected to *L. reuteri* supplementation.

These results are in accordance with other studies involving probiotics as an adjunct to clinical periodontal treatment which reported a marked improvement in patients' clinical condition compared to clinical therapy alone [16–19]. Teughels et al. [17] investigated the effect of *L. reuteri* probiotic lozenges, taken two times per day for 12 weeks, as an adjunct therapy to SRP. The authors observed significantly higher reductions in PD, especially in deep pockets. Significant improvements were observed in our study, especially in terms of probing depth and clinical attachment loss, after the local instillation of *L. reuteri* solution. This aspect is of particular importance, since severe periodontal attachment loss poses a great risk for negative outcomes, e.g., tooth loss among other local and locoregional complications [20,21].

Deep periodontal pockets are also main candidates for surgical therapies [22], therapies which assume greater treatment costs, discomfort, and potential complications [23].

Nevertheless, except Penala et al. [19], who investigated the effects of mouthwash with *L. reuteri* and *L. salivarius*, all studies used chewing tablets as a base for probiotic intake. Local application in periodontal pockets of *L. reuteri* could offer higher benefits due to a delivery system in the particular area of interest, at a higher concentration. At the same time, local administration of the probiotic eliminates a number of concerns about potential unwanted systemic side effects related to the administration of probiotic strains to individuals with systemic pathologies involving mild to moderate immune suppression.

Different strains of *Lactobacillus* proved efficient in decreasing probing depth in moderate periodontal pockets (4–6 mm) compared to placebo + SRP [19,24,25]. Contrary to these data, using *Streptococcus* spp. as a probiotic did not generate the same benefits [26]. This may translate into the fact that not all probiotic strains exhibit the same efficacity. In our study, local delivery of *L. reuteri* DSM 17938 exhibited a probing depth reduction of 0.91 ± 0.23mm, more significant than SRP alone (0.51 ± 0.15mm). Moreover, the clinical attachment gain for *L. reuteri* sites when compared to the SRP alone sites was of 0.99 ± 0.24mm versus 0.37 ± 0.11mm.

Data regarding BOP reduction after probiotic therapy are contradictory. Iwasaki et al. [25] found no significant differences in gingival bleeding between the probiotic and SRP alone groups (reduction of 3.70 and 0.7% for the control and study groups, respectively). Still, their subjects followed periodontal maintenance sessions throughout the study period, a fact that could interfere with the clear impact of probiotics. In our study, BOP reduction in *L. reuteri* sites after five sessions of local delivery was of 65.98% versus 55.27% in control sites, with no proper maintenance program. Tekce et al. [24] and Ince et al. [27] measured BOP one year after consuming *L. reuteri* pills for three weeks without additional SRP and obtained a reduction of 69.6% and 50,95% in the control group and 77.85% and 77.3% in the study group, respectively. Teughels et al. [17] observed a reduced BOP in the study group but did not obtain statistically significant differences in the three-month assessment. Various anti-inflammatory effects have been reported for *L. reuteri*, including the reduction in MMP-8 expression [28] and an increase in TIMP-1, which is a modulator of MMP activity [27]. Moreover, *L. reuteri* proved to be associated with a decrease in proinflammatory cytokines, such as TNF-α, IL-1β, or IL-17 [29]. It also forms reuterin, which induces oxidative stress [27]. This aspect is particularly important if we think of the important influence of periodontal inflammation on the systemic status and the relationship between periodontitis and cardiovascular diseases, osteoporosis, and other conditions and outcomes [30–33].

In terms of microbiological results, one study reported that probiotic pills, either alone or in combination with SRP, decreased the number of *A. actinomycetemcomitans, P. gingivalis,* and *Prevotella intermedia*. Tekce et al. [24] reported greater reductions in periodontal pathogens on days 21, 90, and 180, but did not show any differences between groups on day 360, suggesting that the beneficial effects of probiotics are temporary.

Our study is a pilot one, with certain limitations. First of all, the number of participants is not large enough to extrapolate our results in the general population. Furthermore, our research focused on clinical aspects only, with the main purpose to expand our interests in microbiological and immunological investigations related to the use of *L. reuteri* as adjunctive periodontal therapy. Larger study periods of time are also required to clarify the long-term potential benefit of probiotics. We strongly believe that probiotics could play an important role in the management of particularly severe or resilient cases of periodontitis. However, of course, further studies are required in order to establish a clear work protocol, in terms of particular strains, dosage, number of sessions, and product use. We consider that research should be also conducted in order to obtain a locally delivered product with a controlled release system.

5. Conclusions

Local delivery of *L. reuteri* DSM 17938 associated to conventional non-surgical therapy demonstrated significant improvements of periodontal attachment and a reduction of gingival bleeding in patients with stage 3–4 periodontitis. Further investigations are required to obtain a clear protocol of probiotics as an alternative adjunctive therapy.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the "Grigore T. Popa" UMPh Ethics Committee/30.07.2020.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: All authors share equal contribution.

Conflicts of Interest: The authors declare no conflict of interest.

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