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Probiotics diminish the post-operatory pain following mandibular third molar extraction: a randomised double-blind controlled trial (pilot study)

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RESEARCH ARTICLE

Abstract

The aim of this study was to investigate the effect of a probiotic in preventing infections after third molar surgery. Thirty-eight patients were consecutively enrolled to a double-blind randomised placebo-controlled trial. Patients were asked to take one tablet two times a day containing a mixture of *Levilactobacillus brevis* CECT7480 (KABP-052) and *Lactoplantibacillus plantarum* CECT7481 (KABP-051) or placebo for the first post-intervention week. The primary outcome was the postoperative infection rate. Secondary outcomes included swelling, eating difficulties and postoperative pain recorded by the patient using a visual analogue scale (VAS) during the first postoperative week. No statistically significant difference in the infection rate between the groups was found; with only three cases of infections reported (one in the probiotic group and two in the placebo group) on the first week. Compared to placebo, treatment with the probiotic showed a significantly higher reduction in pain and eating difficulties scores at 5, 6 and 7 days post-surgery. Swelling values were not significantly different between the groups at any time point. The findings of this pilot study justify a larger study to clarify the possible role of these bacterial strains on the post-operative pain management following third molar surgery.

Keywords: lactobacilli, alveolar osteitis, oral surgery, GABA

1. Introduction

Removal of the third molars is one of the most commonly performed procedures in oral surgery and its post-operative recovery requires special care. Pain, swelling, trismus and eating difficulties are usual complications after this procedure. Postoperative infections including surgical site infection and alveolar osteitis are also associated with third molar extractions (Chuang *et al.,* 2008; Sukegawa *et al.,* 2019; Susarla *et al.,* 2003). Keeping the dental plaque under control is crucial to prevent these complications. Various therapies are aimed at minimising the postoperative complications of third molar extraction surgery; of these, the use of systemic antibiotic prophylaxis and local antimicrobial mouthwashes (chlorhexidine) for the prevention of postoperative infections is a widespread practice (Sancho-Puchades *et al.,* 2009). Furthermore, such bactericide agents frequently generate oral and gut microbiota's disturbances and their efficacy remains questionable (Sugano, 2012). Therefore, investigations for providing new strategies to patients undergoing this procedure are becoming increasingly important.

Oral microbiota is known to be the key component of oral health. Numerous studies related the pathogenesis and development of many oral diseases, such as dental caries, gingivitis and periodontitis with changes in the oral microbiota (Bosch *et al.,* 2012; Gruner *et al.,* 2016). Bacteria

account for the main portion of oral microorganisms, with over 600 different species of bacteria commonly found in the mouth (Elavarasu *et al.,* 2012). An imbalance between beneficial and pathogenic bacteria causes the most common humans' oral infections (Anusha *et al.,* 2015; Bizzini *et al.,* 2012; Gupta, 2011).

The use of oral probiotics as a preventive non-invasive approach relays on the concept of beneficial bacteria administration (Gungor *et al.,* 2015). Probiotic organisms compete against pathogenic bacteria and are eventually able to prevail over them (Anusha *et al.,* 2015; Bizzini *et al.,* 2012; Gupta, 2011; Pradeep *al.* 2014). Probiotic supplements can decrease the number of cariogenic bacteria, thus preventing dental caries (Gungor *et al.,* 2013; Twetman and Keller, 2012). Other studies support the probiotic therapy to prevent and treat periodontal diseases and halitosis (Allaker and Stephen, 2017; Invernicci *et al.,* 2018). To our knowledge, the recently published study by Wälivaara *et al.* (2019) is the only one examining the potential benefit of probiotics following third molar surgery, highlighting the limited evidence available to date.

The aim of this study was to evaluate the effect of a probiotic combination of *Levilactobacillus brevis* (formerly *Lactobacillus brevis*) CECT7481 and *Lactoplantibacillus plantarum* (formerly *Lactobacillus plantarum*) CECT7480 in preventing infections in healthy patients after removal of the third molar.

2. Materials and methods

Study design and subjects

This was a single-centre, prospective, double-blind, randomised, placebo-controlled, parallel-group study to evaluate whether probiotic supplementation during 1 week after surgery reduced infectious complications compared to placebo. The study was conducted in accordance with the Helsinki statements, and the protocol was approved by the Ethics Research Committee of Fundació Sant Sant Joan de Déu (Barcelona, Spain) and registered at ClinicalTrials.gov as NCT04203771. Written informed consent was obtained from all adult patients and parents/guardians of patients aged <18 years, before enrolment. All patients were recruited from the Department of Oral and Maxillofacial Surgery at the Hospital HM Nens. HM Hospitales, (Barcelona, Spain), between June 2016 and May 2017.

Healthy subjects of both genders aged between 14 and 25 years who required the surgical removal, with osteotomy, of the two mandibular third molar teeth were considered eligible for inclusion. Exclusion criteria included having gingivitis (gingival index ≥2) or dental plaque (plaque index ≥2), use of probiotics or antibiotics in the previous 30 days and during the study, use of antiseptic mouthwash during study treatment, tobacco use, pregnancy or breastfeeding and allergies to any of the ingredients contained in the active or placebo tablets.

Treatment allocation and concealment

Patients were randomly assigned to receive bucodispersible probiotic tablets (reported mean oral dissaggregation time of 10 min) containing a mixture of *L. brevis* CECT7480 (KABP-052) and *L. plantarum* CECT7481(KABP-051) at a dosage of 5×10^8 cfu for each probiotic strain or to receive placebo, twice a day for 7 days. All tablets had identical aspect, were delivered in identical white containers and were coded according to the computer-generated randomisation list, which was only revealed at the end of the study. Both patients and clinicians were blinded to group assignment.

Surgical procedure and interventions

All patients received a professional oral cleaning prior to the third molar extraction. All surgical procedures were performed by the oral surgery team, with all surgeons having >10 years of experience. Each enrolled subject underwent the same surgical extraction procedure, performed under intravenous sedation and loco-regional anaesthesia (articaine hydrochloride 4% with epinephrine 1:100,000) in the surgical area of our hospital. Standard post-operative instructions were given and all patients were prescribed 20-30 mg/kg/day of oral ibuprofen for the postoperative period of 7 days. In cases of postoperative complications (fever or alveolar osteitis), patients were instructed to take orally amoxicillin 500 mg every 8 h and were withdrawn from the study.

Patients were asked to take 1 tablet of study medication two times a day for the first post-intervention week. Additionally, patients received a postoperative diary after the surgical intervention to record their subjective perception of pain intensity, swelling and eating difficulties on a 10 cm Visual Analogue Scale (VAS) once daily for the first postoperative week.

Two evaluation visits were performed at weeks 1 and 4 of the study for an oral clinical examination and to register the presence and type of postoperative infection. Plaque and gingival status were assessed at baseline and again on week 4 by using Silness and Loe plaque index (PII) and and Loe and Silness gingival index (GI) respectively, on an scale of 0 = no plaque/normal gingiva to 3 = abundance of soft matter/severe inflammation. (Loe and Silness, 1963; Silness and Loe, 1964).

Outcome measures

The primary outcome was the postoperative rate of infectious complications on weeks 1 and 4, as determined by the clinician. Infectious complications were defined as the presence of fever and/or alveolar osteitis. Secondary outcomes included swelling, eating difficulties and pain intensity recorded by the patient in the patient diary during the first postoperative week, as well as plaque and gingival indices determined by the clinical at baseline and week 4. Patients were considered for analysis of swelling, eating difficulties and pain intensity when they returned the patient's diary with information filed for at least 5 out of the 7 days. Safety was evaluated by the incidence of adverse events (AE) and serious adverse events (SAEs) that could be detected by the investigator or communicated by the patient throughout the study.

Analysis neurotransmitter production by bacterial strains

Strains *L. plantarum* CECT7481, *L. brevis* CECT7480, as well as the control probiotic strains *Lacticaseibacillus rhamnosus* GG (ATCC53103) (formerly *Lactobacillus rhamnosus*), *Limosilactobacillus reuteri* DSM19738 (formerly *Lactobacillus reuteri*) and *L. plantarum* 299v (DSM9843) were grown overnight in MRS broth (Difco), at 37 °C under microaerophilic conditions (5%CO_2). Supernatant samples were sterilised through 0.22 µm filters and analysed by HPLC-MS/MS. HPLC was performed in an Agilent 1290 Infinity (Agilent, Santa Clara, CA, USA) with an HS-F5 3 µm column (Supelco Discovery; Supelco, Bellefonte, PA, USA), a mobile phase of H_2O with 0.1% formic acid (A) and MeOH with 0.1% formic acid (B), an injection volume of 2 µl, and a flux of 500 µl/min. Detection of serotonin (5-HT) and γ-aminobutyric acid (GABA) was performed with a 6500 QTRAP mass spectrometer (AB Sciex, Framingham, MA, USA) with IonDriver ESI ionisation in positive mode. Experiments were performed in duplicate.

Statistical analysis

Incidence of infectious complications as alveolar osteitis has been reported to reach up to 30% but is highly variable. Prior data on the effects of study probiotic on post-operative infection rate were not available at the time of protocol design to undertake a sample size calculation. Therefore, we designed this study with an arbitrary sample size of 40 patients to obtain information about the sample size needed to detect a difference in the rate of infectious complications.

Data were checked for normality using the Shapiro-Wilk test. Between-group differences were analysed using Mann-Whitney non-parametric test for discrete and continuous data, while Chi-square test with Pearson's correction for small samples was used for categorical data. Discrete and continuous variables are represented as mean and standard deviation or median and interquartile range (IQR, i.e. the 25% and 75% percentiles). depending on data normality. Categorical variables are described as the number and percentage for each category. Significance was considered at two-sided $P<0.05$. Statistical analysis was performed using IBM SPSS Statistics v20 (Armonk, NY, USA).

Study endpoints were analysed in the per-protocol (PP) population, which comprises all included patients that have at least one efficacy evaluation in at least one follow-up visit. Calculations of statistical power for Mann-Whitney tests were performed with the G*Power software (version 3.1.9, Universität Düsseldorf, Düsseldorf, Germany) (Faul *et al.,* 2007), using the Lehmann method with normal approximation of sum of ranks and a two-sided $P=0.05$ cut-off. Calculation of sample size for a Fisher exact test to achieve 80% power based on the observed rate of infectious complications was performed with the same software, also using a two-sided $P=0.05$ cut-off.

3. Results

Disposition of subjects

Of the 40 patients initially planned, a total of 38 were finally included and randomised to either the probiotic group (n=21) or the placebo group (n=17). A total of 8 patients were excluded from the primary analysis, 4 in each group. The disposition of subjects and the reasons for patient discontinuation by patient group are presented in Figure 1. One of the 38 randomised patient was out of the inclusion criteria age range (13 years old) but was finally included because of slow recruitment rate and the patient actually being close to 14 years old.

Baseline characteristics

Demographics and key baseline characteristics for the subjects recruited in the study are presented in Table 1. Subjects age ranged 13 to 25 years old and 55.2% were female. Overall, baseline clinical data were similar between the two groups, but patients in the probiotic group reported higher eating difficulties total scores than those in the control group.

Primary endpoint

Primary endpoint data were available at week 1 for 18 subjects in the probiotic group and 14 subjects in the placebo group (Figure 1). Only one patient in the probiotic group and two patients in the placebo group developed a post-operatory infection during the first postoperative week, resulting in a 10.0% infection rate in the overall population (5.6% in probiotic and 14.3% in placebo). An additional infection occurred in the probiotic group

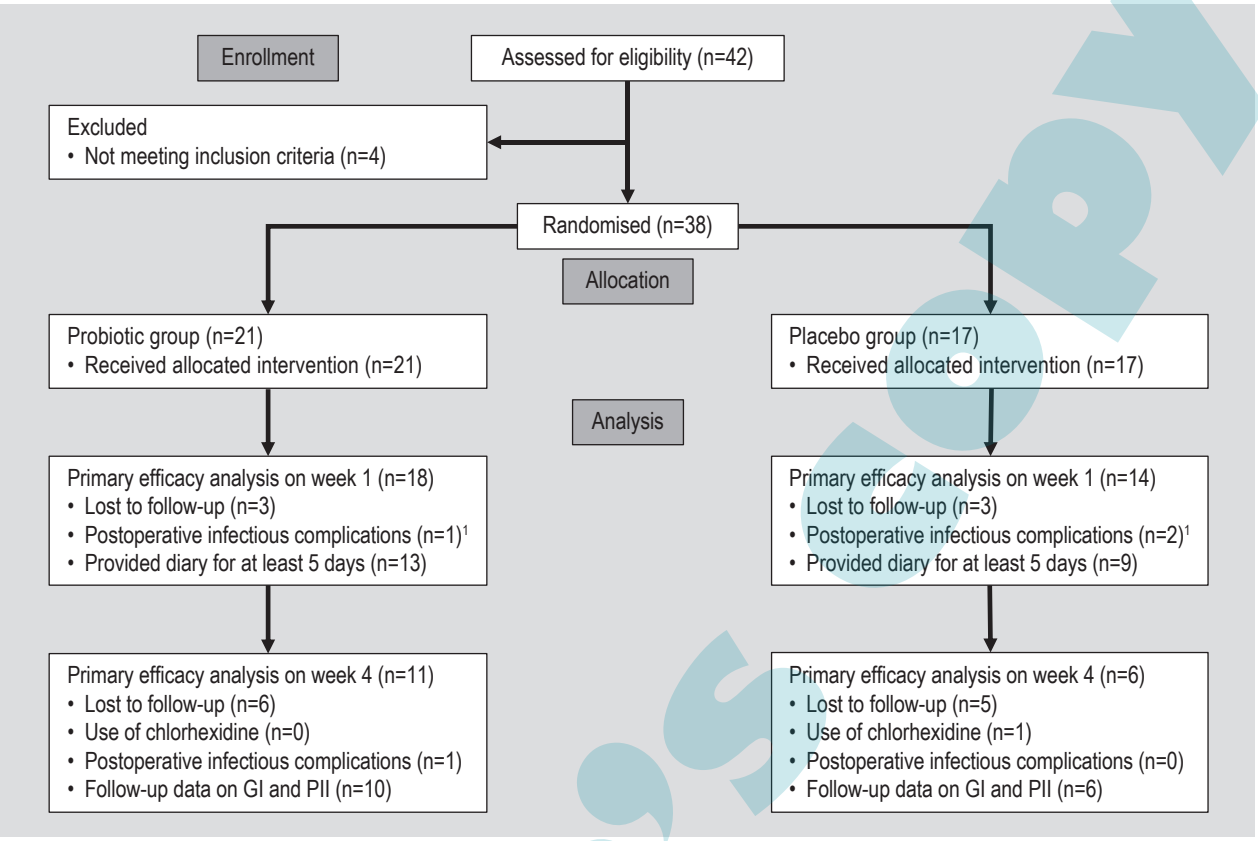

Figure 1. Patient disposition flow-chart. (*) Subjects with infectious complications during week 1 were counted for the primary analysis on week 1 and subsequently removed from the study due to receiving antibiotics, thus not being available for analysis on week 4. Patients available for efficacy analysis at week 1 were those who completed week 1 and attended the corresponding follow-up visit; the same applies to week 4. GI = gingival index; PII = plaque index.

Table 1. Demographic and baseline clinical data.[1]

	Probiotic (n=21)	Placebo (n=17)
Age (years)		
Mean (SD)	16.8 (2.1)	17.4 (3.6)
Gender (female, %)	13 (61.9)	8 (47.1)
Plaque index		
Median (P25, P75)	0.0 (0.0; 0.2)	0.0 (0.0; 0.4)
Gingival index		
Median (P25, P75)	0 (0.0; 0.3)	0 (0.0; 0.5)
Swelling (D1)		
Median (P25, P75)	3 (2; 7.5)	4 (2; 5)
Min, max	0, 10	2, 6
Eating difficulties		
Median (P25, P75)	8 (5; 9)	4 (4; 6)*
Pain (D1)		
Median (P25, P75)	6 (3; 8)	4 (3; 5)

[1] D1 indicates first day (considered baseline point); P25 = 25th percentile; P75 = 75th percentile; SD = standard deviation. * P=0.049 (vs placebo). Variables are expressed as mean (SD) in the case of normal distribution and median (interquartile range) in case of nonparametric distribution.

between postoperative week 1 and 4. None of the patients presented infection after 4 weeks of surgery. There were no statistically significant differences in the cumulative incidence of infectious complications between patients who received the probiotic treatment and those who received placebo, neither at 1 nor at 4 weeks (P>0.10). Using results from week 1, statistical calculations indicate 160 patients per group would be required to reach 80% power to detect the observed difference with statistical significance.

Secondary endpoints

Significant differences were observed in mean changes in pain VAS scores from baseline (Day 1) to consecutive days 5, 6 and 7 post-surgery between the probiotic and placebo groups. On all these post-operative days, subjects in the probiotic group had a higher reduction in pain intensity compared to subjects receiving placebo (P=0.016, P=0.017 and P=0.031, respectively) (Figure 2A). Moreover, the rate of patients achieving a reduction of at least 2 points compared to baseline was significantly higher in the probiotic group than in the placebo on days 5 (9/13 vs 1/9 respectively; P=0.009) and 6 (9/11 vs 3/9; P=0.032) post-surgery, but not on day 7. Similarly, on comparing the reduction of eating

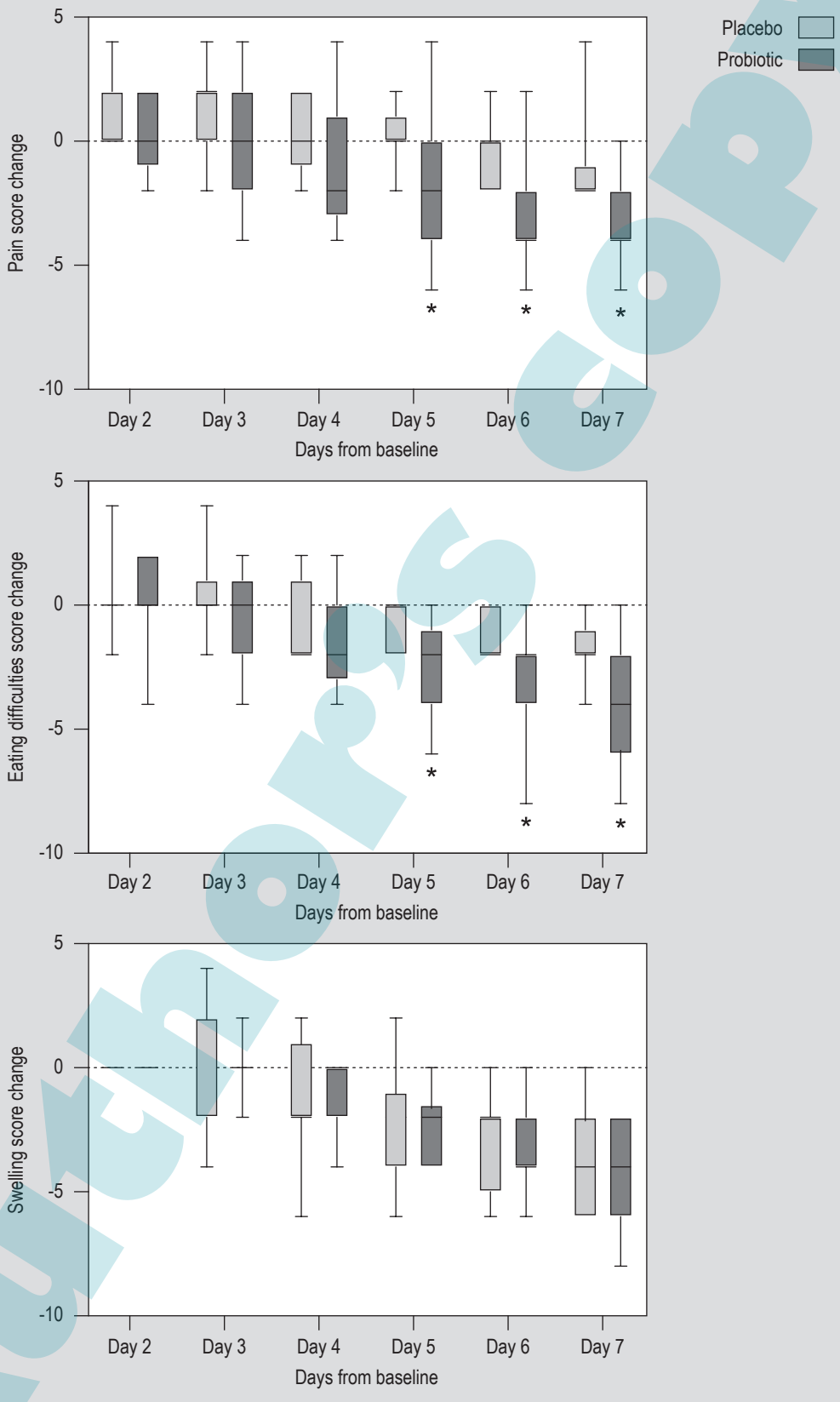

Figure 2. Changes from baseline to day 7 in the visual analogue scale (VAS) score for (A) pain, (B) eating difficulties, and (C) swelling. A reduction in score indicates an improvement. In each box-plot, data is shown as median (line), inter-quartile range (box limits), and min/max (whiskers). * *P*<0.05 (vs placebo).

difficulties on the fifth, sixth and seventh postoperative day, it was found a significantly higher improvement in the probiotic group compared to the placebo group (Figure 2B). Conversely, there were no significant differences between groups in terms of mean swelling changes from baseline at any time point (P>0.10) (Figure 2C).

It should be noted that there were missing data for the three self-reported variables in 5 of 14 patients assigned to placebo and 5 of 18 patients assigned to probiotic treatment, including those patients that received antibiotic treatment due to infectious complications (1 in probiotic and 2 in placebo groups). However, calculation of post-hoc statistical power indicates a 63, 69 and 63% power to detect a difference in pain score change such as observed for days 5, 6 and 7, respectively. Gingival health at week 4 did not show significant differences between treatment groups. The median (25^{th}-75^{th} percentile) PlI values was 0.1 (0.0-0.9) for the probiotic and 0.0 (0.0-0.3) for the placebo group. Similarly, the median GI value was 0.1 (0.0-1.0) for the probiotic group and 0.0 (0.0-0.3) for the placebo group. Regarding safety, only one patient in the probiotic group reported one mild skin eruption which rapidly resolved without medication and was considered not to be treatment-related.

The observed effect on pain improvement against placebo prompted us to analyse the release of GABA and serotonin by probiotic strains *L. plantarum* CECT 7481 and *L. brevis* CECT7480, as well as that of some other widely used probiotic strains. GABA levels were higher in the *L. brevis* CECT7480 strain (2.74 ± 0.05 mM), markedly lower in the *L. plantarum* 299v strain (0.21 ± 0.01 mM) and undetectable in supernatants of other analysed strains. Conversely, serotonin was not detected in any of the strains tested.

4. Discussion

The present study was designed to assess the effect of a probiotic supplement composed of *L. plantarum* CECT 7481 (KABP-051) and *L. brevis* CECT 7480 (KABP-052) in the prevention of infectious complications after the surgical removal of third molar. We found no statistically significant difference in the infection rate between the probiotic and placebo groups, with only three cases of infections were reported during the first week after surgery. However, it was interesting to find that patients in the probiotic group significantly improved the perception of pain and eating difficulties during the second, third and fourth post-operative days. Also, we found the strain *L. brevis* CECT 7480 to be a high producer of GABA. This finding could provide a mechanistic basis for the observed effect and, in our view, deserves further exploration.

The probiotic potential of *L. plantarum* CECT 7481 and *L. brevis* CECT 7480 for improving oral health was previously evaluated in an *in vitro* screening assay. The isolated strains exhibited remarkable antimicrobial activity against oral pathogenic bacteria. Additionally, both strains were resistant to oral conditions, highly adhered to oral tissues and did not present any antibiotic resistance (Bosch *et al.,* 2012). The present study is the first investigating the potential clinical benefit of these two bacterial strains in patients undergoing surgical third molar extraction, a subgroup of patients with common post-operative complications. For example, alveolar osteitis following the extraction of impacted third molars is 10 times more frequent than for other dental extractions, with a reported incidence as high as 30% (Blum, 2002; Rubio-Palau *et al.,* 2015). However, the incidence of postoperative infections varies widely depending on the definition used, the patients characteristics, use of chlorhexidine mouthwash and the surgeon's experience (Almeida *et al.,* 2016; Aravena *et al.,* 2018; Chaparro-Avendano *et al.,* 2005; Garcia *et al.,* 2003; Lopez-Cedrun *et al.,* 2011; Milani *et al.,* 2015; Sukegawa *et al.,* 2019). A lower post-operative complications rate in our study population may also be partly explained by the beneficial healing environment in adolescent patients and their greater capacity in the process of tissue regeneration compared with adults. Given the small number of postoperative infections reported in our study, it is estimated that a much larger sample size (160 per group) would be required for the primary endpoint (rate of infectious complications) in future studies. However, post-hoc sample size calculations reveal a moderate statistical power in pour study to detect differences in pain. In this regard, the validity and generalisability of our results could have been compromised by the small sample size, which limited the number of post-operative infectious outcomes. In a recent study, Wälivaara *et al.* (2019) assessed the effect of *L. reuteri*-containing lozenges on wound healing, swelling, pain and discomfort in 64 patients (18 to 34 years old) undergoing extraction of mandibular third molar. Although the authors found no infectious complications in none of the groups, they argued that, besides the relatively small sample size, the permitted use of chlorhexidine and the different surgical experience of participating clinicians could have also influenced the rate of postoperative oral infections (Reebye *et al.,* 2017; Susarla *et al.,* 2003).

Mandibular third molar surgery is associated with a wide range of symptoms such as pain, swelling and trismus which negatively affect patients' quality of life in the first few days after surgical extraction (Lim and Ngeow, 2017; McGrath *et al.,* 2003). Importantly, patients treated with probiotics in our study showed significantly greater reductions from baseline in pain than those given placebo, suggesting a better analgesic effect of probiotics compared to placebo. The ability of probiotics to reduce pain intensity is probably also responsible for the greater improvements in eating

difficulties in these patients. No significant differences were found regarding swelling scores. In contrast to our findings, Wälivaara *et al.* (2019) observed that patients treated with the probiotic perceived less post-operative swelling compared to placebo, although differences between groups in pain did not reach statistical significance. These disparities may be related to the use of different bacterial strains, which could have differential effects on patient-reported symptoms, as current expert consensus on probiotics point to strain-specificity of many probiotic effects (Hill *et al.,* 2014). Relief of postoperative pain is an essential criterion to assess the overall success of tooth extraction, as pain is one of the most common post-operative complications (Lee *et al.,* 2015). The decrease in pain intensity observed in the test group, although statistically significant for three consecutive postoperative days, should be considered with caution because pain is one of the most subjective symptoms and can vary greatly depending on the mood and physical state of the patient at the time of assessment. Despite that, most of the maxillofacial surgery research studies used the VAS for post-operative pain assessment (Sirintawat *et al.,* 2017), since is a highly reliable tool and more informative and sensitive to measure the change in pain compared to other ordinal scales (Montero *et al.,* 2017).

The assessment of other clinician-reported parameters, such as the gingival and plaque index showed no significant differences between the groups. Therefore, the beneficial effect of this combination of probiotic strains on gingival status or dental plaque could not be demonstrated. Montero *et al.* (2017), recently assessed the same probiotic strains *L. plantarum* CECT7481 and *L. brevis* CECT7480, combined to a third strain (*Pediococcus acidilactici* CECT8633) in the treatment of gingivitis (Sirintawat *et al.,* 2017). In agreement with the present study, they found no significant differences in mean GI between groups; although a significant reduction occurred in the test group regarding the number of sites with severe inflammation. Authors stated that the dilution effect of predominant event (mild gingivitis) may mask the positive effect of the agent on sites with clear signs of inflammation (Montero *et al.,* 2017).

Finally, we should note that the metagenomic study with oral samples to correlate the incidence of postoperative infections with the composition of the oral microbiota was not performed due to the small number of post-operative complications observed and their similar distribution between study groups. Therefore, no conclusions can be drawn about the oral microbiome and their relationship with infectious risk or oral pain after third molar extraction.

As previously discussed, the main limitation of our study lies on the small sample size and low rate of infectious complications, which limited the power to detect some effects. Also, reporting of patient diaries was unexpectedly low. However, despite these limitations, calculations indicate statistical power in the 60-70% range for each of days 5, 6 and 7 to detect an effect of the observed size for pain reduction. However, the distribution of ages was almost identical in the probiotic and placebo groups. Therefore, any bias in pain perception would equally affect both the probiotic and placebo group, and thus does not invalidate our conclusions.

In conclusion, this is the first study evaluating the potential benefits of the probiotic strains *L. brevis* CECT7480 and *L. plantarum* CECT7481 on the post-operative complications following third molar surgery. Interestingly, results showed a significant effect of this supplementation in post-operative pain management compared to placebo which persisted during three consecutive days. However, there were no differences between treatments for the reduction of oral infectious rate. We consider these findings deserve further clinical validation in larger studies to clarify the potential role of these bacterial strains as oral probiotics.

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Conflicts of interest

Jordi Espadaler-Mazo, Ariana Salavert and Meritxell Aguiló-García, are full-time employees of AB-BIOTICS S.A (Sant Cugat del Vallès, Barcelona). All other authors declare no conflict of interest.

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