

# BMJ Open A single-centre investigator-blinded randomised parallel group clinical trial to investigate the effect of probiotic strains *Streptococcus salivarius* M18 and *Lactobacillus acidophilus* on gingival health of paediatric patients undergoing treatment with fixed orthodontic appliances: study protocol

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## ABSTRACT

**Background** There is limited data on the beneficial effects of probiotics on the gingival health of patients undergoing treatment with fixed orthodontic appliances. This study aims to compare the effect of probiotic tablets combined with regular oral hygiene versus regular oral hygiene alone on gingival status in these patients. The effect of probiotic intake on plaque formation and salivary microbiome composition will be also assessed.

**Methods and analysis** This is a 3 month single-centre, single blind (clinical and laboratory examiners), parallel group randomised controlled two arm superiority trial. Fifty paediatric patients attending the Postgraduate Orthodontic Clinic at the Hamdan Bin Mohammed College of Dental Medicine (HBMCDM), Mohammed Bin Rashid University of Medicine and Health Sciences (MBRU), Dubai, United Arab Emirates, who meet the eligibility criteria will be recruited. Block randomisation with 1:1 allocation and concealment of allocation will be carried out. The treatment group will receive probiotic tablets containing *Streptococcus salivarius* M18 and *Lactobacillus acidophilus* together with regular oral hygiene versus the control group on regular oral hygiene alone. Clinical examination and collection of saliva for microbiome assay will be carried out at baseline and end of study. Self-reporting by patients will be used to document acceptability and adverse effects. Statistically significant decrease in gingival bleeding on probing in the treatment group will be classified as primary outcome of treatment success. Statistically significant reduction in Plaque Index, Gingival Index and shift in the composition of the oral microbiome in favour of beneficial bacteria are secondary outcomes indicative of efficacy of probiotic intake.

**Ethics and dissemination** Ethical approval for the study has been granted by the HBMCDM, MBRU, Institutional Review Board (Reference #: MBRU-IRB-2018-015). Study

## Strengths and limitations of this study

- The use of probiotic preparation containing *Streptococcus salivarius* M18, which has known oral health beneficial effect is a strength, it also limits the generalisability of the findings to other probiotic formulations.
- As paediatric patients represent a key population undergoing treatment with fixed orthodontic appliances, targeting these age group is a strength for this study contributing to findings generalisability; however, our results may be less applicable to adults.
- The 3 month duration for administration of the probiotic is longer than that previously reported and provides sufficient time for beneficial effect to become apparent.

findings will be disseminated via publication in peer-reviewed journal.

**Trial registration number** ISRCTN95085398

## INTRODUCTION

### Background and rationale

The risk of enamel decalcification and damage to periodontal supporting tissues are long recognised as problems during orthodontic treatment and remain major concerns, especially in patients with fixed orthodontic appliances.<sup>1</sup> Fixed orthodontic appliances encourage dental biofilm accumulation as bands, brackets, orthodontic

wires and accessories act as plaque traps.<sup>2</sup> Moreover, in this environment meticulous tooth brushing and interproximal care become a much more difficult and time-consuming process that requires skill and dexterity to complete efficiently.<sup>3</sup> Thus, the presence of orthodontic appliances increases biofilm retention and as its accumulation persists, a shift from aerobic to anaerobic supragingival and subgingival periodontal flora occurs and inflammatory responses may be elicited that can be recognised clinically as deterioration in periodontal clinical parameters.<sup>3</sup> It has been shown that this deterioration may only be partially reversed even after 2 years following appliance removal.<sup>4 5</sup> In this respect, daily oral hygiene and monitoring of periodontal tissue status are critical for the success of orthodontic treatment.<sup>2 3</sup>

Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'.<sup>6</sup> *Lactobacillus*, *Streptococcus* and *Bifidobacterium* strains with probiotic properties have been identified. Some of these have been proposed to be beneficial in preventing or treating oral conditions, like caries and periodontal diseases, which are characterised by shifts in the oral biofilm composition, as well as intra-bacterial and bacterial-host interactions.<sup>7-9</sup> Potential beneficial effects of various probiotic strains on oral pathogens have been demonstrated in vitro.<sup>10-12</sup> Reports from studies on the effectiveness of probiotic therapy to prevent or treat gingivitis and periodontitis remain conflicting. While some studies have reported demonstrable efficacy such as reduction of plaque accumulation and gingival inflammation among patients on probiotic products other studies have failed to show similar effects.<sup>13-15</sup> Two recent systematic reviews aimed to determine the effects of probiotics on various periodontal health parameters.<sup>16 17</sup> While a growing number of studies supported the use of probiotic therapy to prevent or treat gingivitis and periodontitis, there were also conflicting findings.<sup>16 17</sup> Moreover, data from short-term studies concerning the clinical effects of probiotics on the gingival health of children and adolescents undergoing treatment with fixed orthodontic is limited.<sup>18 19</sup> In addition, the effect of probiotics on the oral microbiome of patients on fixed orthodontic appliances remains unreported. This randomised clinical trial is designed to address this paucity of data.

All patients on fixed orthodontic appliances are routinely expected to practice daily home oral hygiene and this is the standard of care. In this study, the treatment group will receive probiotics in addition to daily home oral hygiene. They will be compared with the patients practicing daily home oral hygiene alone. The protocol has been registered in ISRCTN registry (ISRCTN95085398).

## Objectives

### Research hypothesis

Gingival bleeding on probing is less in orthodontic patients consuming probiotics in addition to regular oral hygiene, compared with patients practicing daily home oral hygiene alone.

## Primary objective

To compare the effect probiotic tablets together with regular oral hygiene versus regular oral hygiene alone on gingival bleeding on probing of patients undergoing treatment with fixed orthodontic appliances.

## Secondary objectives

1. To compare the effect of taking probiotic tablets together with regular oral hygiene versus daily home oral hygiene alone on the following parameters in patients undergoing treatment with fixed orthodontic appliances:
  - i. Biofilm accumulation on tooth surfaces.
  - ii. Gingival inflammation severity.
  - iii. Salivary microbiome composition.
  - iv. Acceptability of probiotic tablets.
2. To determine the occurrence of adverse effects of the probiotic tablets in patients undergoing treatment with fixed orthodontic appliances.

## METHODS

### Trial design

The proposed study is a single-centre, single blind (to clinical and laboratory examiners), parallel group randomised controlled two arm superiority trial, adhering to the guidelines of the American Dental Association,<sup>20</sup> The Consolidated Standards of Reporting Trials Group<sup>21</sup> and Standard Protocol Items: Recommendations for Interventional Trials reporting guidelines.<sup>22</sup>

### Sample size calculation

The study is sized to have an 80% power to detect a 15% statistically significant difference in gingival bleeding on probing measurements between the two groups.<sup>20</sup> The 15% statistically significant difference in gingival bleeding on probing measurements between the two groups was based on the American Dental Association Acceptance Program Guidelines.<sup>20</sup> The sample size calculations used  $\alpha=0.05$  for a two-sided test, data from an earlier randomised clinical trial<sup>23</sup> and considered a 30% dropout rate in each study arm.<sup>24</sup> Based on this calculation, a total of 50 participants will be recruited for the study.

## PARTICIPANTS, INTERVENTIONS AND OUTCOMES

### Study setting

Subjects in the paediatric age group (up to the 18 years old) will be recruited from patients attending the Postgraduate Orthodontic Clinic at the Hamdan Bin Mohammed College of Dental Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates.

### Eligibility criteria

#### Inclusion criteria

Participants will be individuals, aged under 18 years, planned for simultaneous full arch upper and lower fixed labial orthodontic appliance therapy from second premolar to second premolar. They should be in good

general health and have a dental history that includes brushing at least once a day; be willing and able to comply with the trial regime. In addition, participants will be required to have normal anatomical periodontal attachment and gingival bleeding on probing on at least 30% of the buccal mesial, midline and distal sites of the teeth examined using the criteria for bleeding of the Gingival Index.<sup>25</sup> The rationale for minimum level gingival bleeding on probing is to include subjects in whom some improvement in gingival health would be demonstrable with an effective therapeutic regime following the American Dental Association guidelines.<sup>20</sup>

#### Exclusion criteria

These include inability to obtain informed consent, presence of allergies, sensitivities or food intolerance; any congenital syndromes of the head and neck; medical contraindications such as heart conditions, immunocompromised state or diseases necessitating antibiotic cover prior to procedures, use of immunosuppressants, history of surgery within the past year or planned within the next 90 days; severe nausea, fever, vomiting, bloody diarrhoea or severe abdominal pain within the past 1 month, as well as chronic use of medication of any kind, probiotics or food supplements of any kind and use of antibiotics, anti-inflammatory, steroids or hormones within 1 month before the start of the study. Other exclusion criteria include presence of special physical or mental needs that would compromise manual dexterity; poor compliance with oral hygiene regimens; poor periodontal health (presence of supragingival calculus, subgingival calculus or periodontal pocketing); extensive dental restorations or uncontrolled caries activity; oral prophylaxis in the previous 1 month from start of the study and use of antibacterial mouth rinses or toothpastes with supplementary antibacterial agents within 1 month from the start of the study, pregnancy, smokers and the planned use of premolar bands.

#### Interventions

The two study groups will be those receiving probiotic tablets together with regular oral hygiene (treatment group) and those receiving daily home oral hygiene alone (control group). The study duration will be 3 months. After baseline examination by the clinical team, eligibility verification, participant's assent and parents'/guardian's data submission, participants will be randomised to the two study groups (25 patients per group). Block randomisation with a 1:1 allocation will be performed by the Principal Investigator with the aid of a computer program. Concealment of allocation will be achieved by using identical, sealed, sequentially numbered, opaque envelopes that will contain group assignment and will be prepared by the Principal Investigator. The envelopes will be opened sequentially by a dental assistant, only after the envelope has been irreversibly assigned to each participant and will remain unknown to the clinical team. Participants in the treatment group will be instructed to practice usual oral hygiene and provided with probiotic tablets. The probiotic tablets are commercially available

in the United Arab Emirates and are formulated for oral health in children (Nature's Plus, Source of Life, Animal Parade, Tooth Fairy Probiotic, Children's Chewable, Natural Vanilla Flavour, New York, USA). This probiotic product is formulated with two probiotic strains namely *Streptococcus salivarius* M18 (1 billion CFU) and *Lactobacillus acidophilus* (2 billion CFU). *Streptococcus salivarius* M18 is a probiotic strain specifically beneficial for oral health.<sup>26 27</sup> According to manufacturer instructions, the participants will chew two tablets for 2 min once daily. They will be advised to avoid concomitant use of medication which may affect plaque accumulation for example, antibiotics and antibacterial mouth rinses. If such medication is required on professional advice, they must report this to the Principal Investigator. Individuals in the placebo arm will be instructed to practice usual oral hygiene and will not receive any probiotic supplement. To maintain quality of the clinical trial, unblinding and code breaks will only occur when exceptional circumstances make it imperative that this is essential for further management of the patient. This shall be carried out by the Principal Investigator and reported in the data management system.

The parents of participants in the treatment group will be given 6 weeks supply of probiotic tablets by the dental assistant and will be asked to bring back all unused tablets when picking up the next supply. The daily intake of the tablets will be encouraged by the dental assistant at the patient's regular visits which will be at least once a month. The compliance will be rated as 'acceptable' when  $\leq 3$  tablets per week will have been forgotten and as 'questionable' when this happened more frequently.<sup>28</sup> In addition to being asked about adverse events at each appointment, patients will be asked to stop taking the tablets and report any adverse event immediately if one were to take place.

#### Oral hygiene instructions

After allocation, each patient will receive appropriate oral hygiene instructions by the clinical team. Patients will be asked not to discuss anything related to group allocation with anybody but only with the Principal Investigator. Oral hygiene instructions will include brushing at least for 2 min twice a day, after breakfast and before retiring at night and use of interdental toothbrushes according to the routine home-based instructions given to patients with orthodontic appliances. No timer will be provided because it is not consistent with usual toothbrush home use. No further oral hygiene instruction will be given for the duration of the trial but compliance with the oral hygiene regimen will be verbally reinforced by the clinical team in the patients' follow-up visits. All subjects will be asked to brush with commercially available fluoride-containing dentifrices (1450 ppm F), with no added anti-plaque or anti-calculus ingredients. No other oral hygiene devices, mouth rinses or dentifrices will be permitted during the trial.

### Clinical examination

Data recordings of all patients will take place at baseline and at 3 months (see online supplementary file for data sheet). At each examination the subjects will be asked to not to clean their teeth for 12 to 16 hours (overnight plaque formation).<sup>29</sup> Plaque and gingival health evaluations will then be made. Clinical examinations will be carried out by previously calibrated examiners blinded for the group assignment. To facilitate examination, cheek retractors will be used and cotton wool rolls will be inserted between the upper and lower teeth. The teeth will be then carefully air-dried. Intra-examiner reliability will be checked by comparing two sets of recordings within 1 week. Plaque will be assessed on the buccal surfaces of the teeth with the fixed orthodontic appliances using the orthodontic modification of the Plaque Index which has been shown to be sensitive to detecting differences in plaque levels in orthodontic patients with fixed appliances.<sup>30</sup> Gingivitis will be assessed on the buccal surfaces from second premolar to second premolar using the Gingival Index.<sup>25</sup> Gingival bleeding on probing will be determined as the percentage of the buccal mesial, midline and distal sites of the examined teeth which bleed on probing, using the criteria for bleeding of the Gingival Index.<sup>25</sup> Finally, the clinical examiner will inspect the hard and soft oral cavity tissues for potential adverse effects. Monitoring of tablet safety will be further conducted through verbal questioning at each appointment. Participants will be asked about any adverse event such as fever or gastrointestinal discomfort including nausea, vomiting, bloody diarrhoea or severe abdominal pain. Subjects will be also asked about any changes to their medical history or if any antibiotics had been taken since commencement of the study.

### Patient-reported outcomes

Participants in the treatment group will be asked by the dental assistant to fill up a questionnaire reporting their subjective evaluation of the probiotics. The questionnaire will include the following questions, with three/four closed answers available<sup>31</sup>:

1. How do you feel using the tablets? (great; good; unpleasant; very unpleasant)
2. The taste of the tablets is... (very good; good; fair; bad).
3. Did you use the tablets every day? (yes, one a day; no, I forgot 1 day; no, I forgot 2 days; no, I forgot more than 2 days)
4. How do you feel your teeth after using the tablets? (cleaner and smoother; rough and unpolished; I don't feel any change)

### Saliva sampling and salivary microbiome determination

First collection will be at baseline and the second after 3 months. Saliva samples will be collected after brushing using the OMNIgene Oral, OM-501 tubes (DNA Genotek, Ottawa, Ontario, Canada). These collection tubes have been optimised for microbial sample stability at room temperature storage for up to 6 months. As recommended by the manufacturer, samples will be stored at

room temperature pending DNA extraction and Next Generation Sequencing (see online supplementary file for methodology). Samples will be sent to the laboratory using patient unique study code as identifier. The laboratory will be blinded to the study group allocation.

### Statistical analysis

Data analysis will be conducted using SPSS 24.0.0 (SPSS Inc, USA). As Plaque and Gingivitis Index systems follow an ordinal scale, assigning a number to each of the criteria evaluated, and thus are non-parametric in nature, non-parametric statistical tests will be used. The level of significance will be set  $\alpha=0.05$ .

### Primary outcome measure

Statistically significant decrease in gingival bleeding on probing at the end of the 3 month study period in the treatment group will be classified as treatment success.

### Secondary outcome measure

The following statistically significant outcomes will be classified as evidence of beneficial effect of probiotic intake:

Reduction in Plaque Index; reduction in Gingival Index; shift in the composition of the oral microbiome in favour of beneficial bacteria.

### Other outcomes

Acceptability of the probiotic product will be measured based on the responses to the patient reported outcomes questionnaire.

Adverse effects will be assessed based on self-reporting and clinical examination.

### Data management

Each participant will be assigned a unique study code number. Participant identifiers which will be collected include name, phone number/email address and clinic file number. The list connecting the participant name/contact details with the study code number will be kept in a locked cabinet in the Principal Investigator's office. Only the Principal Investigator will have access to this list. Clinical recording sheets, questionnaires and saliva containers will only be labelled using the code number. All processing in the laboratory will be done using the assigned code number. When the study is completed and the data have been analysed, the list will be destroyed. Data entry and verification will be carried out to ensure accuracy. The findings from the study will be presented at international scientific conferences and submitted for publication in peer-reviewed journals. All authors will be required to have made significant intellectual contributions and no professional writers will be engaged in the manuscript preparation. No personal information will be used in any report or publication.

### Ethics and dissemination

All patients fulfilling the basic inclusion criteria will be eligible for participation. The Principal Investigator will be responsible for identifying those prospective patients who do not fulfil the eligibility criteria from the orthodontic treatment perspective so that they are not approached. Eligible patients will be approached in the

clinic, invited to participate and subsequently examined by the clinical investigator to verify eligibility regarding the periodontal condition and medical history. The purpose of the study will be explained to children and parents/guardians and the consent form will be provided to those who fulfil the periodontal condition and medical history criteria and assent/agree to participate. Children and parents/guardians will be given time to read carefully the consent form. It will be ensured that they fully understand the consent form and any questions raised will be answered. The participants will be expected to assent, that is, affirmatively agree, to participate in the research project and their parents/guardians will provide consent. The researcher obtaining informed consent will not be providing orthodontic treatment in order to avoid any appearance of undue coercion. Sample consent form is provided in the online supplementary file. Confidentiality of collected data will be maintained and data dissemination will be carried out as described above in the data management section.

### Patient and public involvement

We did not involve patients or public in the design of this study.

### DISCUSSION

There is a paucity of data on the effect probiotics on gingival health among patients undergoing treatment with fixed orthodontic appliances. Reported studies evaluating gingival inflammation after short-term use of probiotic (up to 1 month) have failed to show significant difference between the treatment and placebo groups.<sup>18 19 32</sup> The use of probiotics strains which are not known to be beneficial for oral health and the short duration of administration may be contributing factors to the findings of non-efficacy in these studies. As orthodontic patients require rigorous maintenance of oral hygiene to ensure gingival health, determining the efficacy of probiotics through well-designed trials has become imperative. The design of the present study addresses the methodological issues found in previous trials such as those associated with risk of bias. The probiotic product to be used in the present study contains *S. salivarius* M18 which is a strain that has been shown to be beneficial for oral health.<sup>26 27</sup> The administration for probiotics for a short duration of 1 month or less as described in previous trials may be insufficient to enable the probiotic strains to colonise and establish a stable microbiome. In a recent report, microbiological change showing alteration in bacterial composition was detected after 6 weeks administration of oral probiotic preparation.<sup>33</sup> Therefore, in this study, the 3 month duration of administration provides adequate time for the probiotic strains to become established in the oral microbiome and exert efficacious effect. Additionally, our proposed analysis of the taxa and abundance of oral microbiome will provide new information on the

changes in the bacterial composition which have hitherto not been reported in other studies.

This study has also been designed to compare the effect of probiotic tablets combined with regular oral hygiene versus regular oral hygiene alone (which represents the standard of care in patients with orthodontic fixed appliances). To ensure that we keep true to the standard of care, the control patients will not be given placebo tablets and we do not expect this to impact the findings of the study. Although it is recognised that saliva is a key component in maintaining a symbiotic relationship between the host and the oral microbiome, its importance becomes evident in conditions with pathological conditions of decreased salivary flow.<sup>34</sup> The present protocol focuses on healthy individuals who will be instructed to chew two tablets, once daily, for 2 min, a procedure requiring overall much less masticatory effort and thus much less associated saliva secretion. Indeed, studies have shown that even chewing gums two to five times per day, for 10 to 30 min each time, has no significant effect neither on plaque scores nor gingival status.<sup>35–38</sup>

The focus on the paediatric age group could possibly be considered a limitation as the findings might not be generalisable to adults. However, the paucity of data on the clinical effects of probiotics on the gingival health of paediatric patients, who also represent the vast proportion of those undergoing orthodontic care, makes this study relevant despite this limitation. In this study, we have utilised a probiotic preparation which contains a strain known to have beneficial effect on oral health. As the effects of probiotics are strain specific, the findings might not be applicable to other strains. Despite this limitation, the comparison to regular oral hygiene which is standard of care in these patients will provide clinically relevant information. Although beyond the scope of the current protocol, future work in which patients receive different probiotic strains could provide information on the differences in strain efficacy.

In conclusion, the findings of this study will provide evidence-based information regarding the effects of probiotic administration on gingival health in patients undergoing treatment with fixed orthodontic appliances. This could possibly facilitate the prevention of periodontal diseases which have long been recognised as problems during orthodontic treatment and remain major concerns, especially in patients with fixed orthodontic appliances.

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**Contributors** EGK, RN, ACS conceived the study and initiated the study design. EGK, RN, MAH, MK, HH, IH, AS, ACS will be involved in study implementation. AH provided statistical expertise in clinical trial design and will conduct the statistical analysis. SK will provide expertise with data interpretation. EGK, RN, SK, MAH, AH,

ACS are grant holders. All authors contributed to finalising the study protocol and approved the final manuscript.

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**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** Ethical approval for the study has been granted by the Hamdan Bin Mohammed College of Dental Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Institutional Review Board (Reference #: MBRU-IRB-2018-015).

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