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Effects of *Streptococcus salivarius* K12 with nystatin on oral candidiasis—RCT

Lijun Hu | Qinghua Mao | Peiru Zhou | Xin Lv | Hong Hua | Zhimin Yan

Department of Oral Medicine, Peking University School and Hospital of Stomatology, Beijing, China

Correspondence
Zhimin Yan, Department of Oral Medicine, Peking University School and Hospital of Stomatology, 22 South Zhongguancun Avenue, Haidian District, Beijing 100081, China.
Email: yzhimin96@163.com

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Abstract

Objective: To evaluate the efficacy and safety of *Streptococcus salivarius* K12 as an adjuvant in treating oral candidiasis.

Methods: A total of 56 patients were participated in the randomized, double-blinded, placebo-controlled clinical trial. The *S. salivarius* K12 or placebo lozenges plus nystatin tablets were given for up to 4 weeks at 1-week interval and then followed up for 1 week thereafter. We collected and analyzed the mycological and clinical data, treatment course, and safety data.

Results: At the end of the treatment, significant differences were found in the mycological cure rates between K12 group and control group (90.48% and 55.56%, respectively, $p = 0.008$). Survival analysis demonstrated no statistical difference in overall cure rates comprehensively considering mycological cure, clinical improvement, and recurrence ($p = 0.078$), while statistical difference was found in mycological cure ($p = 0.013$) between the two groups. The median treatment courses of K12 group and control group were 3 weeks and 4 weeks, respectively. No severe events were reported during the study.

Conclusion: *Streptococcus salivarius* K12 exhibited potential efficacy and safety as an adjuvant in treating oral candidiasis by enhancing mycological cure and shortening the treatment course of conventional antifungal therapy in this randomized, double-blinded, placebo-controlled clinical trial. Further large-scale clinical studies are desired to accumulate more evidence for its clinical applications.

KEYWORDS
efficacy, oral candidiasis, randomized controlled trial, safety, *Streptococcus salivarius* K12

1 | INTRODUCTION

Probiotics have drawn more and more attention for their beneficial role in health. As one of the commercially available probiotics, *Streptococcus salivarius* K12, also called BLIS (bacteriocin-like inhibitory substance) K12, is isolated from the oral cavity of a healthy child (Tagg & Dierksen, 2003) and used as a kind of oral probiotic on maintaining the health of ear and oral cavity. Previous studies revealed that *S. salivarius* K12 has the ability to interfere with the excessive growth of pathogens occupying the nasopharynx and oral cavity (Wescombe, Hale, Heng, & Tagg, 2012; Zupancic, Kriksic, Kovacevic, & Kovacevic, 2017). Accumulated evidence suggested that it is beneficial for otitis media (Pierro, Colombo, Giuliani, et al., 2016), halitosis (Burton, Chilcott, & Tagg, 2005; Masdea et al., 2012), and acute pharyngotonsillitis (Pierro, Colombo, Zanvit, & Rottoli, 2016) possibly due to its antimicrobial

activity and immunomodulatory properties. In addition to producing bacteriocin-like inhibitory substances as the lantibiotic salivaricin A2 and salivaricin B (Hyink et al., 2007; Wescombe, Heng, Burton, Chilcott, & Tagg, 2009) do, K12 strain also plays an active role in the host defense process, such as inhibiting the NF-kB pathway to down-regulate inflammatory responses (Cosseau et al., 2008).

Apart from the application in oral diseases such as halitosis, in 2012 Ishijima SA reported that *S. salivarius* K12 could protect the mice from oral *Candida* infection, indicating that *S. salivarius* K12 played a protective role against oral candidiasis (Ishijima et al., 2012). Oral candidiasis is common in AIDS patients, xerostomia, and diabetes, and in those on antibiotics or immunosuppressant and even in those who are denture wearers or with poor oral hygiene (Berberi, Noujeim, & Aoun, 2015; Ellepola & Samaranayake, 2000; Lamster, Lalla, Borgnakke, & Taylor, 2008; Shinozaki et al., 2012). Currently, antifungal agents such as nystatin, fluconazole, and miconazole are frequently used to treat oral candidiasis (Niimi, Firth, & Cannon, 2010), but the subsequent resistance of *Candida* species and the side effects limit their application (Lopez-Martinez, 2010). Therefore, *S. salivarius* K12 is worth to be further explored as a new adjuvant for oral candidiasis.

Although probiotics such as *Bifidobacterium longum*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* do have a role against oral candidiasis (Li et al., 2014; Miyazima, Ishikawa, Mayer, Saad, & Nakamae, 2017), there is currently a lack of evidence on clinical application of *S. salivarius* K12 in oral candidiasis. Thus, this study aimed to assess the efficacy and safety of *S. salivarius* K12 in combination with nystatin in treating oral candidiasis. Its potential and applicability in the improvement of oral candidiasis will be of essential clinical significance.

2 | MATERIALS AND METHODS

2.1 | Study registration

The study was approved by the Human Research Ethics Committee of Peking University Health Center (PKUSSIRB-201412024) in June 2014, and the randomized controlled trial (RCT) was registered with the Chinese Clinical Trial Registry (ChiCTR-TCR-14005090). All subjects signed written informed consent before participation. The study followed the CONSORT statement (Moher et al., 2012; Schulz, Altman, & Moher, 2010).

2.2 | Study design and patients

This study was a randomized, double-blinded, placebo-controlled clinical trial aimed to evaluate the efficacy and safety of *S. salivarius* K12 in combination with nystatin in treating oral candidiasis. The subjects were outpatients of Peking University School and Hospital of Stomatology from September 2014 to June 2018. The inclusion criteria were as follows: (a) 18 years and older, gender is not limited; and (b) oral candidiasis diagnosed on the basis of clinical symptoms/signs and laboratory tests (smear fungal test and/or saliva's fungal culture). The exclusion criteria were as follows: (a) chronic mucocutaneous candidiasis or systemic fungal infections; (b) use of systemic antifungal agents and antibiotics within 1 month prior to participation or use of topical antifungal agents (except for use of Daktarin or nystatin cream or suppository for the treatment of vaginal candidiasis) within 2 weeks prior to participation; (c) an allergic or intolerable history of the *S. salivarius* K12 lozenges; (d) pregnant or lactating women; (e) HIV infection; (f) uncooperative patients for a psychological history or other disorders; (g) abnormal liver and kidney function; and (h) participation in other clinical trials within 1 month prior to participation.

2.3 | Randomization and blinding

All the patients were randomly allocated into two groups (K12 or control) using a sequence generated by computer using SAS software (SAS Institute Inc). Allocation concealment was achieved via sequentially numbered, sealed, opaque envelopes. Both the subjects and investigators who distributed the *S. Salivarius* K12/placebo lozenges to the subjects were blinded to the interventions used. The placebo had similar appearance, size, and color but without *S. salivarius* K12.

2.4 | Sample size

In previous study, the mycological cure rates of probiotics for treating oral candidiasis in the probiotics group and control group were 90% and 65%, respectively (Li et al., 2014). For the purpose of achieving 80% power with 10% significance level ($\alpha = 0.05$; $\beta = 0.20$; both one-sided) in this study, and considering the withdrawal rate of 20%, the trial required 38 subjects for each group.

2.5 | Interventions

The patients in the K12 group received the *S. salivarius* K12 lozenges (≥1 × 10^9 CFU of *S. salivarius* K12 per lozenge, BLIS K12®, Alaron Products Limited) with the regimen of 1 lozenge BID and nystatin tablets (500,000 U, Zhejiang Zhenyuan Pharmaceutical Co., Ltd) with the regimen of topical administration (TID). Patients in the control group received placebo lozenges and nystatin tablets with the same regimen. The patients were required return visits on the 7th, 14th, 21st, and 28th day from then. The end point of treatment was defined as the negative laboratory testing (by smear test and saliva's fungal culture). The last follow-up visit was 1 week after the end of the treatment.

2.6 | Collection and culture of oral specimens

One milliliter unstimulated saliva was collected from each subject. For the saliva collection, subjects were asked not to consume solid food or liquids or to perform any oral hygiene procedures for at least 2 hr before collecting saliva. Then, 0.5 ml sample was incubated at

37°C for 48 hr on CHROMagar plates (CHROMagar) at baseline and on Sabouraud Dextrose Agar (BioMérieux) at return visits on the 7th, 14th, 21st, and 28th day. Two researchers counted the number of colony-forming units (CFUs) per milliliter of saliva by visual inspection.

2.7 | Outcome measures

2.7.1 | Primary outcomes

Clinical cure

Complete remission of clinical symptoms and signs. The self-reported clinical symptom scores were recorded: 0 score represented no symptoms; 1 score represented mild symptoms; 2 score represented moderate symptoms; and 3 score represented severe symptoms. The signs of removable white plaque or erythema were scored based on the area of oral lesions: 0 score represented no lesions; when the area of the lesions were less than 0.5 cm^2 or between 0.5 and 1 cm^2, 1 score and 2 score were given, respectively; and 3 score was given if the area of the lesion was more than 1 cm^2.

Mycological cure

Demonstrated by negative microscopy results by using 10% potassium hydroxide and no growth of *Candida* in culture. This outcome is distinct from the clinical cure in that it does not require the demonstration of the normal appearing.

The treatment end point was determined as the complete eradication of *Candida* species (mycological cure).

2.7.2 | Secondary outcomes

Patient responses were also assessed by median treatment courses and recurrence (clinical and mycological) 1-week after the end of treatments.

The overall cure was defined as mycological cure, marked improvement (60% or more) of clinical symptoms and signs, and no recurrence in the follow-up visit 1 week after the end of the treatment.

2.7.3 | The safety evaluation

During the study period, any symptoms and objective findings of the subjects were recorded, and abnormal changes were monitored

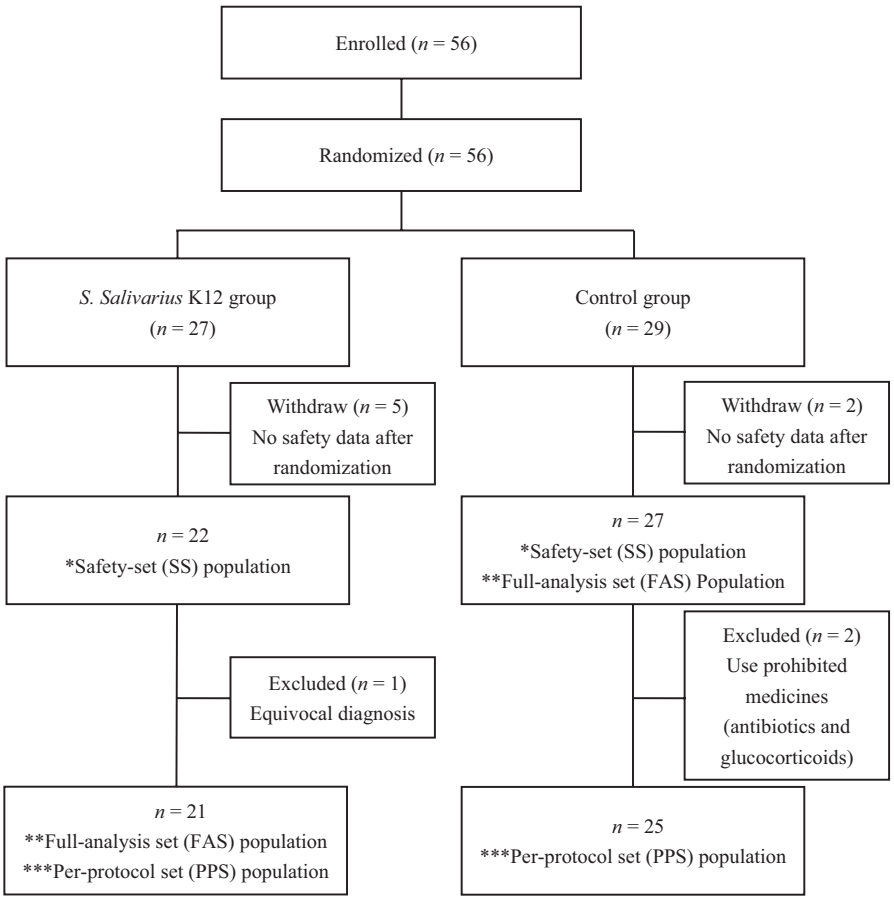

FIGURE 1 Patient randomization and disposition. *For safety analyses, the safety-set (SS) population is used. Safety population includes all randomized patients who take at least one dose of the study medicine. **The full-analysis set (FAS) population is a trial population, that is, as close as possible to the general population for which a test treatment is intended. The FAS population can include individuals who fail to comply with the treatment protocol. *** The per-protocol set (PPS) population is the subset of the FAS population that adhered to the treatment protocol, and consequently, excluding individuals who fail to comply with the treatment protocol

TABLE 1 Demographics of subjects

Baseline characteristics	*Streptococcus salivarius* K12 group (*n* = 21)	Control group (*n* = 27)	*p* value
Age (years, mean ± *SD*)	61.15 ± 10.227	66.19 ± 12.081	0.124
Gender (M/F)	3/18	4/23	1.000
Underlying diseases[a] (Yes/No)	14/7	21/6	0.390

[a]Underlying diseases include cardiovascular diseases such as hypertension and angina, endocrine diseases such as diabetes and hyperthyroidism, digestive diseases such as gastritis, and immune system diseases such as rheumatoid arthritis.

TABLE 2 Baseline characteristics of the full-analysis set population who had oral candidiasis

Baseline	*Streptococcus salivarius* K12 group (*n* = 21)	Control group (*n* = 27)	*p* value
Oral *Candida* counts (lg CFU/ml; mean ± *SD*)	2.37 ± 1.15	2.75 ± 0.81	0.221
Pain			
No (0)	12 (57.14%)	17 (62.96%)	0.584
Mild (1)	5 (23.81%)	7 (25.93%)	
Moderate (2)	4 (19.05%)	3 (11.11%)	
Severe (3)	0 (0%)	0 (0%)	
Burning			
No (0)	15 (71.43%)	14 (51.85%)	0.323
Mild (1)	2 (9.52%)	9 (33.33%)	
Moderate (2)	4 (19.05%)	4 (14.81%)	
Severe (3)	0 (0%)	0 (0%)	
Pseudomembrane			
No (0)	20 (95.24%)	27 (100%)	0.257
Mild (1)	1 (4.76%)	0 (0%)	
Moderate (2)	0 (0%)	0 (0%)	
Severe (3)	0 (0%)	0 (0%)	
Erythema			
No (0)	0 (0%)	1 (3.70%)	0.737
Mild (1)	9 (42.86%)	11 (40.74%)	
Moderate (2)	8 (38.10%)	11 (40.74%)	
Severe (3)	4 (19.05%)	4 (14.81%)	

to identify the occurrence of adverse events (AE) for the safety evaluation.

2.8 | Statistical analysis

SPSS 22.0 (SPSS Inc) was utilized to perform statistical analysis. The data analysis and the reporting of the results of the trial followed the CONSORT guidelines. The differences in baseline data between the *S. Salivarius* K12 group and the control group were analyzed using chi-squared test, *t* test, and nonparametric tests. The improvement of clinical symptoms/signs between the two groups was analyzed by nonparametric tests. Chi-squared test and survival analysis were used to analyze the differences in the cure rates and treatment course of the two groups. All included patients in the safety analysis were received at least 1 dose of study medication.

3 | RESULTS

3.1 | Subject disposition

Patients were recruited over four years of period. Because of recruitment difficulties, in total, 56 patients participated in the study, including 27 patients in K12 group and 29 in control group. Figure 1 summarized the patient disposition.

3.2 | Baseline characteristics

No significant differences were found between the K12 group and the control group with regard to age, gender, and underlying diseases (Table 1). Table 2 lists the baseline CFU counts of fungal culture and clinical symptoms/signs scores of subjects, and it showed

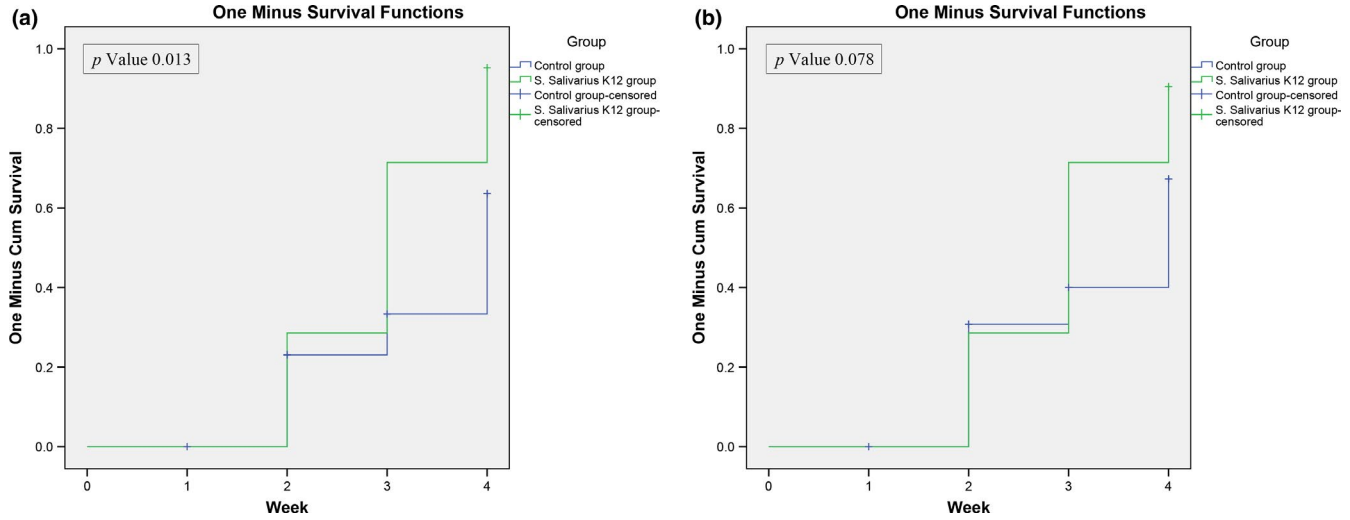

FIGURE 2 (a) Kaplan–Meier survival analysis of the studied groups considering the mycological cure. (b) Kaplan–Meier survival analysis of the studied groups considering the overall cure [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Comparison of the clinical therapeutic efficacy between the two groups

Symptoms/signs	*Streptococcus salivarius* K12 group	Control group	*p* value
Pain			
Alleviate (1)	8 (88.89%)	9 (90.00%)	0.968
Unchanged (2)	1 (11.11%)	1 (10.00%)	
Aggravate (3)	0 (0%)	0 (0%)	
Total	9	10	
Burning			
Alleviate (1)	5 (83.33%)	10 (76.92%)	0.831
Unchanged (2)	1 (16.67%)	3 (23.08%)	
Aggravate (3)	0 (0%)	0 (0%)	
Total	6	13	
Pseudomembrane			
Alleviate (1)	1 (100%)	0 (0%)	--
Unchanged (2)	0 (0%)	0 (0%)	
Aggravate (3)	0 (0%)	0 (0%)	
Total	1	0	
Erythema			
Alleviate (1)	20 (95.24%)	23 (88.46%)	0.413
Unchanged (2)	1 (4.76%)	3 (11.54%)	
Aggravate (3)	0 (0%)	0 (0%)	
Total	21	26	

no significant difference between the K12 group and the control group. Besides, the *Candida* species distribution of the subjects was listed in the Table S1.

3.3 | Efficacy analysis

The mycological cure rates of the K12 group and the control group during the study period were 90.48% and 55.56%, respectively, and significant difference was showed between the two groups (p = 0.008). Survival analysis demonstrated that there was statistical difference in mycological cure rate between K12 group and control group (p = 0.013, Figure 2a), while no statistical difference was found considering overall cure (comprehensively considering mycological cure, marked improvement of clinical symptoms and signs, and recurrence in the follow-up visit) (p = 0.078, Figure 2b). Besides, the median treatment courses of K12 group and control group were

TABLE 4 Summary of overall safety data

Adverse events	*Streptococcus salivarius* K12 group (*n* = 22)	Control group (*n* = 27)	*p* value
Oral discomfort	Xerostomia (*n* = 1), numbness (*n* = 1), burning (*n* = 1)	Xerostomia (*n* = 4), burning (*n* = 1)	0.654
Gastrointestinal discomfort	Borborygmus and pharyngeal discomfort (*n* = 1)	Nausea (*n* = 1)	0.885
Neuropathic symptoms	Light dizziness (*n* = 1), light headache (*n* = 1)	Light dizziness (*n* = 1)	0.445
Skin symptoms	--	Erythra (*n* = 1)	0.372

3 weeks and 4 weeks, respectively. And at the time points of 3 and 4 weeks during the treatment, both the overall and mycological cure rates of the K12 group were all higher than that of the control group (Figure 2a and 2b).

From the clinical aspect, at the end of treatment, no significant differences were noted in the remission of clinical symptoms/signs between K12 and control groups (Table 3). What we observed was that when the mycological cure was achieved, the subjects in both groups maintained improving clinically even after ceasing the treatment.

The photographs of pretreatment and post-treatment were supplied in the Figure S1. After treatment, the symptoms of erythema and atrophy of papilla improved in both K12 and control groups.

3.4 | Safety analysis

A total of 14 patients, 6 in the K12 group and 8 in the control group, reported adverse events during the study (Table 4). In the safety profile, no statistical difference was found between the two groups ($p > 0.05$). No severe adverse events occurred in both groups. One patient complained borborygmus and pharyngeal discomfort in K12 group, and it was considered a possible drug-related adverse event.

4 | DISCUSSION

Streptococcus salivarius K12 appears to have excellent evidences as a kind of oral probiotic with more than a decade of tradition (Zupancic et al., 2017). It is reported that *S. salivarius* strains inhibit the biofilm formation of *Streptococcus mutans* and suppress potentially detrimental upper respiratory tract bacteria, such as *Streptococcus pneumonia* and *Streptococcus pyogenes* in vitro (James & Tagg, 1991; Ogawa et al., 2011; Tamura et al., 2009; Wescombe et al., 2009). Also, some clinical trials and retrospective observational studies supported that *S. Salivarius* K12 was effective for secretory otitis media, recurrent tonsillitis and/or pharyngitis and hemolytic streptococcus pharyngo-tonsillar infections (Gregori et al., 2016; Di Pierro, Adami, Rapacioli, Giardini, & Streitberger, 2013; Di Pierro, Pasquale, & Cicco, 2015).

However, the interaction and mechanism between *S. Salivarius* strains and fungi of *Candida* remain unclear. In a study focus on the in vitro effect of *S. Salivarius* K12 on the excessive growth of *Candida albicans*, the author found that *S. salivarius* K12 was not directly antifungal but preferentially bound to hyphae rather than yeast to suppress *Candida* adhesion to the plastic substratum, and it was not correlated with the antimicrobial activity of the bacteriocin (Ishijima et al., 2012). Then in vivo, a study indicated that *S. salivarius* K12 protected the mice from oral candidiasis (Ishijima et al., 2012). Although the existing studies indicated that *S. salivarius* K12 have the potential against oral *Candida* growth, it is noteworthy that there is a lack of clinical evidence in humans on *S. Salivarius* K12 for the treatment of oral candidiasis. For this purpose, a randomized, double-blinded, placebo-controlled trial was designed to assess the efficacy as well as safety of *S. salivarius* K12 lozenges as an adjuvant in treating oral candidiasis.

The main findings of this study were *S. salivarius* K12 exhibited potential efficacy as an adjuvant in oral candidiasis by increasing the mycological cure rate and shortening the routine treatment course of antifungal therapy with nystatin. It is found that the mycological cure rate of the K12 group was significantly higher than control group, suggesting that *S. salivarius* K12 might prevent the *Candida* colonization and/or promote *Candida* eradication. This finding from clinical trial is consistent with what previously reported in the vitro and animal study (Ishijima et al., 2012). It is with no doubt that treatment with a shortened course has the potential to greatly reduce antifungal medication use in regions of oral candidiasis, with anticipated cost savings and improved compliance. Moreover, shortened course of antifungals may protect the patients from developing drug-resistant strains and decrease the likelihood of adverse events.

Another interesting phenomenon is that there was a statistical difference in cure rate considering mycological cure between K12 group and control group, while no statistical difference in clinical cure rate between the two groups. A possible inference is that remission of clinical symptoms/signs lags behind mycological elimination, which was consistent with what was observed from clinic. Besides, the current scoring system (0–3) of clinical symptoms and signs is not delicate enough, although it is the commonly accepted method for evaluating clinical manifestations of oral candidiasis. In the future, a modified scoring system with more practicability and accuracy is desired for clinical study.

With respect to the safety of *S. Salivarius* K12, no severe adverse event occurred in the K12 and control groups. Although

short-term adverse events reported involving the oral cavity, gastrointestinal tract, and even the nervous system (dizziness) and skin, no significant difference was showed compared with the placebo control group. In K12 group, one patient complained borborygmus and pharyngeal discomfort and it was considered a possible *S. Salivarius* K12 lozenges-related adverse event. The safety findings are consistent with what Burton JP *et al*. and other clinical studies reported on the use of *S. salivarius* K12 in the treatment of oral malodor and streptococcal pharyngitis (Burton, Chilcott, Wescombe, & Tagg, 2010; Burton et al., 2011; Burton, Wescombe, Moore, Chilcott, & Tagg, 2006; Burton, Chilcott, Moore, Speiser, & Tagg, 2006; Gregori et al., 2016; Masdea et al., 2012; Di Pierro et al., 2013, 2012; Di Pierro, Colombo, Zanvit, Risso, & Rottoli, 2014). However, further investigations are still needed for the detection of long-term and rare adverse events.

This study has some limitations that should be considered. Firstly, the insufficient number of subjects related with recruitment difficulties had impact on the reliability of the results. We strictly implemented every step of the study accordance with the study protocol to provide some useful and interesting information or clue for the clinicians and future studies. Further clinical studies are also necessary to confirm these findings in a larger-scale, multicenter context. Secondly, since this is the first clinical study of *S. Salivarius* K12 on oral candidiasis, few references can be obtained. We reviewed the similar clinical studies of other species of probiotics on oral candidiasis as the basis of sample size calculation (Li et al., 2014), which may have influence on the assessment of efficacy. Thirdly, owing to the follow-up period was relatively limited, the maintenance of efficacy was not able to be ascertained. Finally, the possible mechanisms of *S. salivarius* K12 against *Candida* infection and the changing in oral microflora before and after the application of *S. salivarius* K12 need to be explored in the future.

5 | CONCLUSION

Streptococcus salivarius K12 exhibited potential efficacy and safety as an adjuvant in treating oral candidiasis by enhancing mycological cure and shortening the treatment course of conventional antifungal therapy in this first randomized, double-blinded, placebo-controlled clinical trial. Further large-scale clinical studies are desired to accumulate more evidence for its clinical applications.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Zhimin Yan and Hong Hua designed the study. Lijun Hu, Qinghua Mao, Xin Lv, and Peiru Zhou conducted the research. Lijun Hu and Zhimin Yan analyzed the data. Lijun Hu drafted the paper. Zhimin Yan revised the manuscript.

ORCID

Lijun Hu https://orcid.org/0000-0001-5196-5759
Zhimin Yan https://orcid.org/0000-0002-0751-7374

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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