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Research Article

An Investigation into the Intake of A Postbiotic – Salavans® on the Improvement of Well-Being on Adults with Self-Reported Throat Discomfort: A Pilot-Scale Study

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Abstract

The complex composition of microbial community in oropharynx makes the etiology and pathology of pharynx discomfort and illness complicated and recalcitrant. Regular antibiotic treatment manifests several limitations such as antibiotic resistance and disease recurrence. In previous in vitro research, a postbiotic named Salavans®, screened and selected from over 800 lactic bacteria, could aggregate and compete with Streptococcus pyogenes (S. pyogenes), which is the common and dominant cause of pharyngitis and tonsilitis. In our study, we conducted a clinical trial by orally supplementing Salavans contained formula to individuals with self-reported pharynx discomfort for 7 days. The discomforts including coughing, dryness, itchiness and so on were relieved while no adverse events were reported. By characterizing the microbial community composition in the pharynx using 16s rDNA sequencing, it was observed that the relative abundance of certain pathogenic bacteria genera such as Streptococcus, Neisseria and Prevotella were significantly reduced. Therefore, it is concluded the administration of Salavan not only could reduce the specifically targeted Streptococcus but also could alter the microbiota composition in pharynx to a healthy state.

Introduction

Most people have suffered from the poorly and imprecisely defined "sore throat", which actually refers to the inflammation of the pharynx called pharyngitis. In the United States, there were over 15 million acute pharyngitis outpatients visiting to the health providers [1]. Patients usually suffer from a variety of symptoms including sore throat, high fever, nausea, vomiting and so on [2]. Except for factors like virus and smoking, group A b-haemolytic streptococcus especially Streptococcus pyogenes (S. pyogenes) contributes to 5-30% the overlapped infectious and non-infectious disease in children and adults [1]. In population with no obvious symptoms, 10% to 25% of them actually carry group A b-haemolytic streptococcus [3].

The pathology of bacterial pharyngo-tonsillitis and acute otitis media occurs while Streptococcus pyogenes interact with humans through carriage over mild and superficial infections mucosal membranes and then to a systemic and purulent manifestations [4]. The pathogen particular prefers the mucosal membranes of oropharynx and intact skin [5]. Once adhering to these cells, S. pyogenes would internalize into them and form very recalcitrant biofilms, which protects pathogens from antibiotic treatment and leads to recurrent infections [6-7]. Besides, the complex pathogenicity also comes from various factors including age, time, underlying diseases, environment and so on. For instance, the infectious risk was high in both elderly and male subjects especially in environmental conditions such as insufficient household size and overcrowding that would aggravate the transmission of S. pyogenes [8].

The traditional treatment for S. pyogenes infections lies in the topical dermal or mucosal decolonization of this pathogen by systematic or local antibiotics, which adversely cause antibiotic resistance and intolerance [9]. Except for antibiotic treatment, a few clinical studies have shown the efficacies of microbial antagonism of S. pyogenes [10]. For instance, Streptococcus salivarius or placebo were orally administered to children in kindergarten for 6 months. The episodes of streptococcal pharyngitis and acute otitis media during the 6-month trial and 3-month follow-up were significantly reduced while no side-effects were observed [11]. The eradication of the overgrowth of pathogenic bacteria is due to the release of the antibiotic salivaricin A2 and salivaricin B from Streptococcus salivarius [12]. However, probiotics might not be perfect when the treatment is combined with antibiotics. For instance, no statistically significant differences in clinical evaluations were observed when supplementing S. salivarius to patients with acute pharyngotonsillitis concurrently treated with penicillin [13].

Recently, a postbiotic called Salavans® has been screened out from over 800 lactic acid bacteria and impressively demonstrated to eradicate S. pyogenes by directly competing with the binding of S. pyogenes to oropharynx epithelial cells and by aggregating planktonic S. pyogenes that are not yet binding and internalized into those cells. The new mechanisms of this postbiotic makes it promising and potential as an alternative treatment for pharyngotonsillitis. However, beyond in vitro studies, there is limited data of this postbiotic demonstrating its effectiveness in human trials. In this study, a pilot-scale trial was conducted in subjects with self-reported throat discomfort to investigate its effectiveness and the local microbiota diversity and shift in oropharynx.

Materials and Methods

Testing Product

The testing product called Salavans® tablets was manufactured and provided by Shanghai Topvita Nutriceutical Industry Co., Ltd. The product of the synergistic formula consists of postbiotic Salavans® and three Chinese traditional herbal extracts including honeysuckle, chrysanthemum and monk fruit. Salavans® is a patented inactive Lactobacillus crispatus, which also passes the QPS with no any report of pathogenic genes or adverse event.

Study Design/Intervention

The study was carried out as a single-blinded, randomized and self-controlled trial on the effects of 20 subjects (8 female and 12 female) after 7 days of oral intake. All participants signed consent agreement that manifests their benefits from this test and relevant risks. Before the inception of the study, all testing protocols and consent agreement were scrutinized. The participants were supplemented with 3 tablets a day between meals for 7 consecutive days. The advantage of a tablet is the slow release of the postbiotic that is extremely crucial to function in mucosal areas of throat.

Cohort

Healthy subjects from 28 to 45 years old who reported to have the following symptoms in the past month before the test or in a chronic manner: burning, dryness, lumping, itchiness, soreness and inflammation; they are capable of reading Chinese and comprehending the consent agreement; they are willing to cooperate and complete all the tests during the study and report any adverse events. Any subjects who had a cold or fever during the recruitment were excluded and kept following their doctors' advice or prescriptions.

Subjective Assessments of The Treatment

At the completion of the study, the subjects were asked to finish an online questionnaire consisting of 8 questions. Each question is relevant to the improvement in throat symptoms with scores from 0–10 (Score 01-no improvement, scores 2-4 - a general improvement, scores 5 to 8 – obvious improvement, scores 9 to 10 – thorough improvement). The definition of improvement efficacy is the percentage of subjects who scored over 1. During the whole study, the participants were requested to report instantly to the investigator once they experienced any uncomfortable feelings.

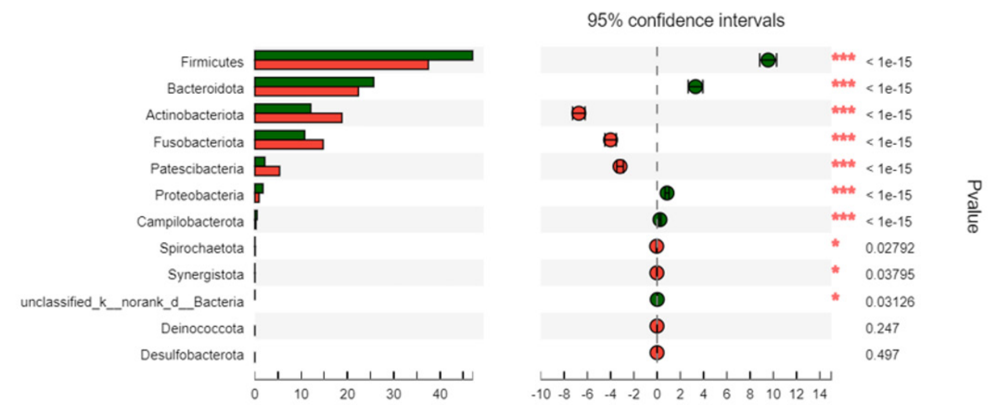
16S rDNA Gene Sequencing and Data Analysis

Pharyngeal swab was performed at the beginning and completion of the test. The samples were collected in the sterilized tubes in a dry ice filled box and transferred to Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) within 4hours. The microbiota diversity of the oropharynx was performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. 2% agarose gel electrophoresis was used to detect the extraction quality of DNA and the bands with OD260/280 between 1.8 and 2.0 were taken for the follow-up test. The extracted DNA was the template and the V3-V4 region of 16S rRNA gene was amplified with forward primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Then, the amplified gene sequence was sequenced on the Illumina MiSeq platform (Illumina, San Diego, USA, Figure 1) and analyzed on the I-Sanger platform (Majorbio, Shanghai, China).

Figure1: Illumina MiSeq.

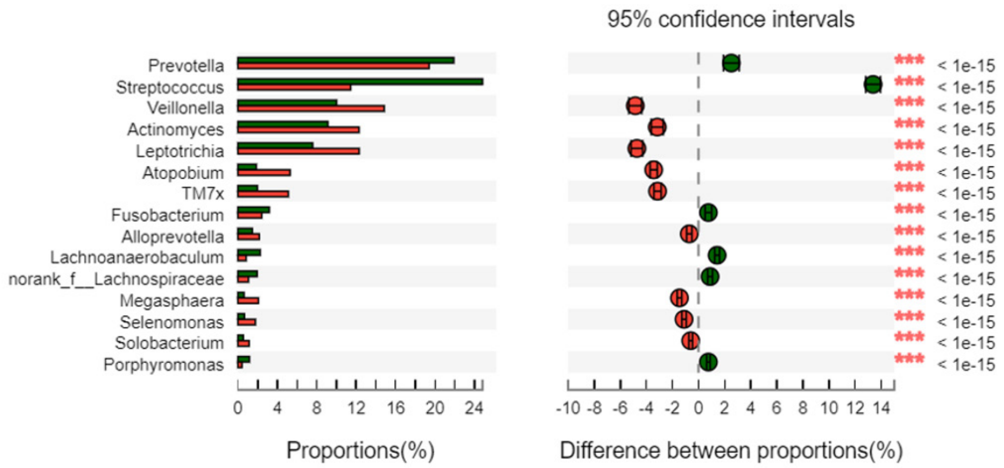
Results

The subjective assessment of the supplement from 8 aspects is shown in the Table 1. The overall efficacy of the treatment is 91%, indicating a substantiate percentage of subjects who had improvement regarding dryness, coughing, discomfort, throat smoothness, inflammation, bad breath, voice and foreign matters. Some subjects did not have improvement in certain aspects because they do not have such symptoms. The microbiome composition profiles of pharynx were established via 16S rRNA gene sequencing based on the average relative abundance assigned to the phylum, class and genus levels. The dominant bacteria at phylum level are Firmicutes, Bacteriodota, Fusobacteria, Actinobacteria, Patescibacteria, Proteobacteria and Campylobacteria (Figure 2). At genus level, it is clearly observed that the relative abundance of Prevotella, Streptococcus, Neisseria are reduced (Figure 3).



Note: the green and red bars represent the relative abundances of bacteria before and after the treatment respectively

Figure 2: Fisher's exact test bar plot on phylum level.



Note: the green and red bars represent the relative abundances of bacteria before and after the treatment respectively.

Figure 3: Fisher's exact test bar plot on genus level.

Table 1: Subjective assessment of treatment.

Presenting Symptoms	Though Improvement	Obvious Improvement	General Improvement	No Improvement	Efficacy (%)
Dryness/Itchiness	5	10	8	2	92
Coughing	10	5	9	3	88
Discomfort	9	8	5	3	88
Throat smoothness	13	7	3	2	92
Inflammation	16	4	4	3	88
Bad breath	11	5	5	4	84
Voice	12	8	2	3	88
Foreign matters	11	9	3	2	92
Overall	87	56	39	22	89

Discussion

The disadvantages of using antibiotics to treat oropharynx discomfort and disease make probiotic and postbiotic as promising treatment alternatives. In our human trial, the administration of the postbiotic Salavans® for 7 days improves the quality of life from aspects of dryness, inflammation, coughing and so on. We hypothesized the main reason of this positive advances is due to the reduction of S. pyogenes, because Salavans® was able to aggregate S. pyogenes in vitro in the preliminary research. To demonstrate this in vivo, we conducted 16s rDNA sequencing test in pharynx mucosa area and found the relative abundance of Streptococci could be reduced after administration of the product for 7 days.

The microflora in the oropharynx is highly associated with oropharynx health and can be causative in infections and inflammations once there is a dysbiosis. Except for Streptococci, the indistinguishable progression of the illness from mild throat discomfort to classic exudative inflammation with high fever might result from other species as Fusobacterium necrophorum, Arcanobacterium haemolyticum and Neisseria gonorrhoeae [14]. As culturing might not show most of the underrepresented bacteria or unculturable bacteria, our 16s rDNA sequencing showed advantages for characterizing the microbial community by drawing a whole picture of oropharynx before and after the treatment. We hypothesized the administration of Salavans® would shift the microbial flora to a state that favors the cure of oropharynx discomfort.

In our 16s rDNA sequencing, it is not difficult to see the dominant bacteria at phylum level are firmicutes, bacteriodota, fusobacteria, actinobacteria, patescibacteria, proteobacteria and campylobacteria, which are commonly found in human nostril and oropharynx [15]. At genus level, the relative abundances of several potential pathogenic bacteria such as Fusobacteria were reduced. Strong scientific evidence has shown that Fusobacterium necrophorum, a gram-negative anaerobe, plays a causative and etiologic role in non-streptococcal tonsillitis and pharyngitis and even suppurative complications and life-threatening condition likes Lemierre's syndrome in adolescents and adults if not adequately treated, which in the United States has exceeded those related to pharyngitis induced by A beta-hemolytic streptococcal [16-17]. For tonsillitis patients, a dysbiosis in the tonsillar microbial flora occurs, in which an increasing predominance of Fusobacterium necrophorum could aggravate the etiology with the development of an inflammatory response [18].

It was found cigarette smoking created anaerobic conditions favoring the proliferation of anaerobic microorganisms such as Fusobacterium [19]. The surface and core microbiota in the tonsils of chronic tonsillitis (CT) and tonsillar hypertrophy (TH) using 16S rRNA gene sequencing of V3-V4 regions showed a higher relative abundance of bacterial genera, including Haemophilus, Streptococcus, Neisseria, Capnocytophaga, Kingella, Moraxella, and Lachnospiraceae [G-2] in patients with TH and Dialister, Parvimonas, Bacteroidales [G-2], Aggregatibacter, and Atopobium inpatients with CT [20]. In our trial, the relative abundances of Neisseria and Prevotella were reduced. Prevotella, implicated in various oral conditions such as periodontitis [21] and endodontic abscesses [22] was found enriched in tonsilitis patients [23] and possible worsen the disease through the production of cytotoxic end products [24,25].

Also, it can be observed in our trial that the relative abundance of this bacterium was reduced. Research has shown that antibiotics prescribed to individuals in the tonsillitis cohort did not alter the microbial community composition [23]. Therefore, except for potential risks such as antibiotic resistance, this kind of treatment may not deal well with oropharynx infections complicated by varieties of bacteria while the treatment of postbiotics can alter the microbial flora composition to a healthier state and thus treat streptococcal and non- streptococcal related oropharynx discomfort and illness. However, the exact mechanism remains to be investigated in the future.

Futuristic Plans

Although our investigation of postbiotics on human subjects provide valuable information, here are several futuristic research plans. First, the number of the subject cohort is small to interpret the data precisely. Besides, the exposure to antibiotics of the subjects in a short or medium term was not strictly monitored, which would affect the microbiota during the intervention. Furthermore, microbial genera in oropharynx consists of multispecies in their phylogenetic classifications; however, amplifying part of the 16S rRNA gene (V3-V4 regions) might not provide accurate, high resolution and reliable taxonomic classification at the species level. Therefore, the identification of those pathological species is needed in the futuristic studies. The limitation of the 16s rDNA technology might also produce contrast results.

Conflict of Interest

The authors declare there is no conflict of interest.

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