

## The Use of *Streptococcus salivarius* K12 in Attenuating PFAPA Syndrome, a Pilot Study

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### Abstract

PFAPA (Periodic Fever with Aphthous stomatitis, Pharyngitis, and Adenitis) syndrome is a rare, poorly understood, clinical entity characterized by a sudden onset of fever, aphthous stomatitis, pharyngitis, and cervical adenitis. PFAPA affects mainly children and generally resolves before puberty with no subsequent consequences for the patient. Use of acetaminophen (APAP), anti-inflammatory drugs, corticosteroids and tonsillectomy are considered potential treatment options. *Streptococcus salivarius* K12 is a perfectly tolerated, oral-colonizing probiotic strain that releases two lantibiotics (salivaricins A2 and B) known to antagonize the *in vitro* and *in situ* growth of many oral pathogenic streptococci, as well as being endowed with anti-viral and anti-inflammatory capabilities. As demonstrated in our study the 90-day administration of strain K12 appears to have reduced specific signs of PFAPA, leading also to a reduction in drug use. Larger prospective and controlled studies are now indicated to more definitively establish the relevance of our assumption and the importance of these preliminary observations.

**Keywords:** PFAPA syndrome; *Streptococcus salivarius* K12; IL-8; Inflammatory cytokines; Children

### Introduction

Periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA) represents the most common periodic fever syndrome of childhood. The etiology is poorly understood. No genetic defects have yet been identified and the incidence of the syndrome has not been accurately established [1]. The condition is characterized by a sudden onset of fever and can be accompanied by aphthous stomatitis, pharyngitis, and cervical adenitis with less common symptoms including headache, rash, and gastrointestinal disturbances. PFAPA episodes normally recur every 3 to 8 weeks and resolve within 6-7 days of onset [2]. Even if published supportive evidence is limited and its precise role has yet to be clarified, tonsillectomy appears to have been the most common option for management of PFAPA syndrome when symptoms markedly interfere with the child's quality of life and/or other strategies, such as treatment with acetaminophen and anti-inflammatory drugs, has failed [3]. Although the pathogenesis remains obscure, the disease displays characteristics of an inflammatory reaction. During PFAPA attacks, complement, IL-1-related and IFN-induced genes are significantly overexpressed. PFAPA flares are also accompanied by significantly increased serum levels of chemokines for activated T lymphocytes, GM-CSF, G-CSF, and pro-inflammatory cytokines such IL-1 $\beta$ , IL-6 and IL-8 [4,5]. The probiotic bacterium *Streptococcus salivarius* K12 produces the lantibiotic bacteriocins, named salivaricin A2 and salivaricin B [6]. These bacteriocins interfere with the growth of *Streptococcus pyogenes*, *Haemophilus influenza*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*, bacteria commonly involved in the pathogenesis of bacterial pharyngotonsillitis and/or acute otitis media (AOM) [7]. Recent clinical trials, conducted in children have demonstrated that treatment with

probiotic K12 reduces the occurrence of episodes of pharyngotonsillitis and AOM [8-10] without modifying the levels of either IL-1 $\beta$  or TNF- $\alpha$ , but with considerable reduction of IL-8 release [7]. We have therefore assumed that the strain K12, being endowed with anti-pharyngitis, anti-tonsillitis and anti-inflammatory properties, could counteract PFAPA syndrome. Indeed, some anecdotal reports of parents of PFAPA-affected children that the characteristic symptoms of PFAPA appeared to have abated while their children were taking probiotic K12 has encouraged us to propose the hypothesis of a possible anti-PFAPA effect played by the strain K12.

### Methods

*Streptococcus salivarius* K12 (BLIS Technologies, Dunedin, New Zealand), was formulated as slowly-dissolving oral tablets by SIIT (Trezzano S/N, Milan, Italy) and notified as nutritional supplement to the Italian Ministry of Health as Bactoblis<sup>®</sup> by Omeopiacenza (Pontenure, Italy), according to the provisions of law 169 of 2004, on July 5, 2011 (notification number: 53435). The preparation of Bactoblis<sup>®</sup> used contained no less than 1 billion colony-forming units (CFU)/tablet of *Streptococcus salivarius* K12. Starting from T=0 to T=90, 1 tablet of Bactoblis<sup>®</sup> was administered to each subject every night, just before sleep. The tablet was allowed to slowly dissolve in the oral cavity, without biting or swallowing. Saliva production is typically reduced in the evening hours and this improves the effectiveness of oral colonization. Only for the very first treatment, the administration of the tablet was preceded, about 30 minutes before, by the use of a chlorhexidine-based (0.2%) mouthwash. This procedure improves the efficacy of oral colonization by creating bacteria-depleted niches in the oral tissues. In order to evaluate the level of subject adherence to the established protocol, the subjects were asked to return any unused product boxes and tablets. Acceptable adherence was considered to be the administration of not less than 95% of the allocated tablets. Our

study concerning the PFAPA-affected children treated with the strain K12 has been performed with both ethical committee positive opinion and parental consent. Because of the small number of subjects tested, no statistical analysis has been performed.

## Results

The four children evaluated in our work and diagnosed with PFAPA syndrome were all outpatients of the Pediatric Department at Bambino Gesù Hospital (Rome, Italy).

As shown in Table 1 their principal PFAPA-relevant features were: 1) one fever episode, with temperature of about 39°C, per month; 2) one pharyngitis episode per month; 3) possible presence of aphthous stomatitis and/or cervical adenitis; 4) possible use of cortisone and/or ibuprofen and/or acetaminophen; 5) poor quality of life and sleep. As shown in Table 2, three subjects out of four had no episodes of fever,

aphthous stomatitis, pharyngitis or lymphadenopathy during 90-days treatment with the strain K12. Consequently, no drugs were administered during this period. According to their parents, for these three subjects both quality of life and sleep also improved substantially. Just a bit different results were reported for a fourth subject (Table 3) with no signs consistent with those of PFAPA syndrome occurring during the second and the third month of treatment with strain K12. For this subject one day of fever occurred during the first month of treatment and one day of fever occurred during the following 30-day wash out period. Also for this fourth subject, both life and sleep quality were reported by the parents to be improved. This fourth case seems to be a very infrequent presentation of PFAPA syndrome and might be differently explained, especially as related to the impressive amount of medication (apparently ineffective) prescribed during the first month of observation.

Subject; age (years) and sex	PFAPA diagnosis (month/year)	Fever episodes (per month); maximum temperature; duration of fever	Presence of aphthous stomatitis	Pharyngitis episodes per month; duration of pharyngitis	Presence of cervical adenitis	Cortisone use	APAP use; times per day	Ibuprofen use; times per day	Quality of Life (1-10)	Quality of Sleep (1-10)
1; 5; male <sup>oo</sup>	01/2015	1.0; 39.5°C; 4 days	yes	1.0; 3 days	no	no	yes; 4	yes; 4	2	2
2; 10; male <sup>^</sup>	11/2013	1.5; 39.0°C; 4 days	no	1.0; 1 day	yes	yes	yes; 4	yes; 4	3	3
3; 6; male <sup>o</sup>	09/2014	1.0; 39.0°C; 4 days	no	1.0; 4 days	yes	no	yes; 4	yes; 4	5	7
4; 7; male <sup>*</sup>	01/2014	1.0; 39.5°C; 5 days	yes	1.0; 4.5 days	yes	yes	yes; 3-4	yes; 3-4	2	2

<sup>oo</sup> APAP: 250 mg/dose; Ibuprofen 150 mg/dose; <sup>^</sup>Deflazacort 32 mg/dose/die; APAP 500 mg/dose; Ibuprofen 200 mg/dose; <sup>o</sup>APAP 250 mg/dose; Ibuprofen 150 mg/dose; <sup>\*</sup>Deflazacort 30 mg/dose; APAP 500 mg/dose; Ibuprofen 200 mg/dose

**Table 1:** Clinical conditions and parameters of the four cases of PFAPA prior to their use of probiotic K12.

Parameters	1° month with K12	2° month with K12	3° month with K12	4° month (wash out)
Fever episodes; maximum temperature; duration of fever	No fever	No fever	No fever	No fever
Presence of aphthous stomatitis	No lesions	No lesions	No lesions	No lesions
Pharyngitis episodes; duration of pharyngitis	No pharyngitis	No pharyngitis	No pharyngitis	No pharyngitis
Presence of cervical adenitis	No lymphadenopathy	No lymphadenopathy	No lymphadenopathy	No lymphadenopathy
Use of cortisone	No use	No use	No use	No use
Use of APAP	No use	No use	No use	No use
Use of Ibuprofen	No use	No use	No use	No use
Quality of Life (1-10) <sup>o</sup>	7.0 ± 1.0	8.0 ± 1.5	10.0 ± 0.0	9.0 ± 0.5
Quality of Sleep (1-10) <sup>o</sup>	7.0 ± 1.0	7.0 ± 1.0	7.0 ± 1.0	7.0 ± 0.5

<sup>o</sup> average values ± standard deviation are given

**Table 2:** Signs and symptoms trends in subjects 1, 2 and 3 during the 90 days treatment with probiotic K12.

Parameters	1° month with K12	2° month with K12	3° month with K12	4° month wash out
Fever episodes; maximum temperature; duration of fever	1; 40°C; 2 days	No fever	No fever	1; 39°C; 1 day
Presence of aphthous stomatitis	No lesions	No lesions	No lesions	No lesions
Pharyngitis episodes; duration of pharyngitis	1; 4 days	No pharyngitis	No pharyngitis	1; 1 day
Presence of cervical adenitis	No lymphadenopathy	No lymphadenopathy	No lymphadenopathy	No lymphadenopathy
Use of cortisone*	1 day	No use	No use	1 day
Use of APAP°	2 days	No use	No use	No use
Use of Ibuprofen^	2 days	No use	No use	No use
Quality of Life (1-10)	4	7	8	8
Quality of Sleep (1-10)	4	7	7	7

\*Deflazacort: 30 mg/dose/die; °APAP: 500 mg x 3-4 doses/die; ^Ibuprofen 200 mg x 3-4 doses/die

**Table 3:** Signs and symptoms trends in subject 4 during the 90 days treatment with probiotic K12.

## Discussion

Although the pathogenesis of the PFAPA syndrome has not been clarified it appears to be associated with the onset of an inflammatory cytokine cascade. *Streptococcus salivarius* K12, also identified as ATCC BAA-1024, in addition to being effective in counteracting some of the bacteria and viruses implicated in pharyngo-tonsillitis and OMA, has also been shown to be effective in reducing the levels of some cytokines, such as IL-8, that are involved in the development of the inflammatory response and possibly in the pathogenesis of PFAPA. We have anecdotally observed that the use of the strain K12 seems to interfere with the development of signs and symptoms of PFAPA syndrome, thereby limiting the use of drugs and improving the quality of life and sleep of children having a diagnosis of PFAPA syndrome. Nevertheless PFAPA syndrome is not yet precisely defined but even presents aspects so polymorphic in their repetitiveness (duration of febrile episodes, age of onset, associated symptoms) to be easily confused with other conditions such as recurrent infections related to life in community, recurrent bacterial infections favored by recent viral infections, cyclic neutropenia or transient IgA deficiency. These results seem to confirm our hypothesis and suggest a possible option that can result in the reduction of the use of non-steroidal anti-inflammatory drugs, steroids and/or antibiotics, as well as possibly eliminate the use of tonsillectomy for a disease that beyond the discomfort for the patient and for the family is anyways absolutely benign. Since PFAPA syndrome is a disease with hyper-immune responses involving different tissues, the most probable starting point would be the involvement of tonsillar lymphoid tissue which is possibly stimulated by bacterial and viral infections. We predict that prolonged use of *Streptococcus salivarius* K12 would have a favorable effect. Our observations are very preliminary but provide encouragement for further investigation of the use of probiotic K12 for the control of this syndrome. Considering the absolute tolerability of the product, double-blind, randomized, prospective, placebo-controlled studies are now indicated to systematically evaluate the efficacy of probiotic K12 in the control of PFAPA syndrome.

## Conflict of interest

FDP, as Velleja Research Scientific & Research Director, is the formulator responsible of the finished product containing the strain K12. The other Authors report no conflict of interest.

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## References

- Esposito S, Bianchini S, Fattizzo M, Baggi E, Marchisio P, et al. (2014) The enigma of periodic fever, aphthous stomatitis, pharyngitis and adenitis syndrome. *Pediatr Infect Dis J* 33: 650-652.
- Vigo G, Zulian F (2012) Periodic fevers with aphthous stomatitis, pharyngitis, and adenitis (PFAPA). *Autoimmun Rev* 12: 52-55.
- Garavello W, Pignataro L, Gaini L, Torretta S, Somigliana E, et al. (2011) Tonsillectomy in children with periodic fever with aphthous stomatitis, pharyngitis and adenitis syndrome. *J Pediatr* 159: 138-142.
- Stojanov S, Lapidus S, Chitkara P, Feder H, Salazar JC, et al. (2011) Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and Th1 activation responsive to IL-1 blockade. *Proc Natl Acad Sci U S A* 108: 7148-7153.
- Ling E, Israeli M, Klein T, Amir J, Harel L (2014) Periodic fever, aphthous stomatitis, pharyngitis and cervical adenopathy syndrome is associated with activation of GM-CSF and burst-like expression of IL-8 in peripheral blood. *Mod Rheumatol* 24: 997-1000.
- Hyink O, Wescombe PA, Upton M, Ragland N, Burton JP, et al. (2007) Salivaricin A2 and the novel lantibiotic salivaricin B are encoded at adjacent loci on a 190-kilobase transmissible megaplasmid in the oral probiotic strain *Streptococcus salivarius* K12. *Appl Environ Microbiol* 73: 1107-1113.
- Wescombe PA, Hale JD, Heng NC, Tagg JR (2012) Developing oral probiotics from *Streptococcus salivarius*. *Future Microbiol* 7: 1355-1371.
- Di Pierro F, Donato G, Fomia F, Adami T, Careddu D, et al. (2012) Preliminary pediatric clinical evaluation of the oral probiotic *Streptococcus salivarius* K12 in preventing recurrent pharyngitis and/or

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- tonsillitis caused by *Streptococcus pyogenes* and recurrent acute otitis media. *Int J Gen Med* 5: 991-997.
9. Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS (2014) Use of *Streptococcus salivarius* K12 in the prevention of streptococcal and viral pharyngo-tonsillitis in children. *Drug Healthc Patient Saf* 6: 15-20.
10. Gregori G, Righi O, Risso P, Boiardi G, Demuru G, et al. (2016) Reduction of group A beta-hemolytic streptococcus pharyngo-tonsillar infections associated with use of the oral probiotic *Streptococcus salivarius* K12: a retrospective observational study. *Ther Clin Risk Manag* 12: 87-92.