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BRIEF REPORT

Preliminary investigations of the colonisation of upper respiratory tract tissues of infants using a paediatric formulation of the oral probiotic *Streptococcus salivarius* K12

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Abstract A powder preparation of the oral probiotic *Streptococcus salivarius* K12 has been given to 19 young otitis media-prone children following a 3-day course of amoxicillin administered as a preliminary to ventilation tube placement. In two subjects, the use of strain K12 appeared to effect the expansion of an indigenous population of inhibitory *S. salivarius*. In other children, strain K12 colonisation extended beyond the oral cavity to also include the nasopharynx or adenoid tissue. The relatively low proportion (33%) of subjects that colonised was attributed to failure of the amoxicillin pre-treatment to sufficiently reduce the indigenous *S. salivarius* populations prior to dosing with strain K12 powder.

Otitis media is an all too frequently occurring bacterial disease afflicting children worldwide. Persistent episodes can result in either the need for surgical intervention or serious long-term sequellae, such as hearing loss [1]. Probiotics have been increasingly promoted as an alternative to antibiotics prophylaxis to reduce the occurrence of the disease [2, 3]. Prominent amongst the candidate probiotics due to their efficacy in interfering with the growth of major respiratory tract pathogens in vitro are members of the alpha-haemolytic streptococcal species *Streptococcus mitis*, *Streptococcus sanguinis* and *Streptococcus oralis* [2]. Unfortunately, however, each of these species has been relatively commonly implicated in the development of infective endocarditis [4]. More recently, the non-haemolytic *Streptococcus salivarius* K12, a bacterium shown to have low pathogenic potential [5], has been widely used as an oral probiotic for the maintenance of oral health and the control of halitosis. The potent inhibitory activity of strain K12 has been attributed to its production of several bacteriocins, including the lantibiotics salivaricin A2 and salivaricin B, encoded by a 180-kb transmissible megaplasmid [6, 7]. The currently available strain K12 products principally comprise of freeze-dried cells compressed into lozenges, a format unsuitable for infants due to the potential for choking. In the present study, a powdered paediatric formulation has been evaluated for its efficacy in colonising the upper respiratory tracts of young children.

The powdered formulation, containing maltodextrin, xylitol and freeze-dried strain K12 cells, was provided by BLIS Technologies Ltd. (Dunedin, New Zealand). The product was tested for stability at 4°C and 25°C over 6 months and showed no detectable loss of viability at 4°C, while at 25°C, the colony-forming units per gram dropped from 1.7×10^{10} to 5.0×10^{9}. Before commencement of the study, ethical approval was obtained from the University of Otago Ethics Committee. Nineteen subjects (age range 6 months to 5 years) were recruited from a group of patients scheduled for ventilation tube placement at the Dunedin Public Hospital. Two weeks prior to surgery, the enrolled subjects were treated with amoxicillin (125 mg, twice daily for 3 days) to effect a temporary reduction in the levels of their native oral streptococcal populations in order to facilitate subsequent colonisation by the strain K12 cells in the probiotic formulation. Following the antibiotic pre-

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treatment, a teaspoonful (equivalent to ca. 1 g of powder) of the strain K12 preparation was applied to the child's tongue surface twice daily by a parent or guardian on each of the 10 days prior to surgery. Tongue swab samples were obtained just prior to the initiation of the antibiotics treatment and, at the time of surgery, swab samples of both the tongue and nasopharyngeal microbiotas were obtained. In some cases (and with consent), an adenoid tissue sample was also obtained. The presence of streptococci was specifically determined by plating the samples onto mitis-salivarius agar (Becton, Dickinson and Company, Baltimore, MD). The levels of colonisation with the probiotic strain were initially estimated using simultaneous antagonism tests, by determining the proportion of the typical (large, soft and pale blue) *S. salivarius* colonies present in the mitis-salivarius cultures that, when tested as stab cultures, produced wide zones of inhibition in the lawn cultures of indicator strain I1 (*Micrococcus luteus*) and indicator strain I3 (*Streptococcus anginosus*) [8]. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping [9] of representative strongly inhibitory colonies was then used to distinguish those colonies having the characteristic ERIC profile of strain K12 from other strongly inhibitory *S. salivarius* that may have been present in the subjects' microbiota prior to strain K12 administration. For further characterisation of representative isolates, PCR amplification was used with primers specific for the structural genes of the strain K12 lantibiotics SalA2 and SalB [7].

S. salivarius inhibitory to indicators I1 and I3 (and PCR-positive for both SalA2 and SalB) were detected in the pre-colonisation tongue samples of subjects 2, 8, 9 and 15 (Table 1). However, only the isolates from subject 8 had an ERIC-PCR identical to that of strain K12. Following application of the strain K12 colonisation protocol, 10 of the 19 subjects were found to harbour strongly inhibitory *S. salivarius*. PCR testing showed K12-like ERIC profiles for all of the representative strongly inhibitory post-colonisation isolates from the six subjects who had initially harboured inhibitor-negative *S. salivarius* populations. The responses to dosing with strain K12 differed in each of the four subjects who had prior populations of strongly inhibitory *S. salivarius*. In subject 2, there was no boost in the low proportion of inhibitory *S. salivarius*. Subject 8 already had a population of K12-like bacteria (based on ERIC profiles) and their proportion of K12-like *S. salivarius* increased substantially following exposure to strain K12. Subject 9 also had a predominant population of strongly inhibitory *S. salivarius*, but these were distinctive from K12 by ERIC and their relative proportion in the total population did not appear to change following exposure to strain K12. Subject 15 displayed a large increase in the proportion of their pre-dosing population of non-K12-like strongly inhibitory *S. salivarius*. This apparent stimulation of population expansion of prior-established BLIS-producers resembles the findings of an earlier study where the exposure of children to an SalA-producing *S. salivarius* was shown to evoke a marked increase in the proportion of

Table 1 Colonisation of the upper respiratory tract with *Streptococcus salivarius* K12

Subject	Pre-colonisation			Post-colonisation								
	Oral			Oral			Nasopharynx			Adenoid		
	A	B	C	A	B	C	A	B	C	A	B	C
1	+	0	ND[1]	+	18	+	+	0	ND	+	74	+
2	+	6	-	+	7	-	-	ND		+	0	ND
3	+	0	ND	+	95	+	+	98	+	+	14	+
4	+	0	ND	+	0	ND	-	ND		-	ND	
5	+	0	ND	+	0	ND	-	ND		-	ND	
6	+	0	ND	+	0	ND	+	0	ND	-	ND	
7	+	0	ND	+	52	+	-	ND		+	48	+
8	+	10	+	+	54	+	-	ND				
9	+	94	-	+	84	-	-	ND				
10	+	0	ND	+	0	ND	-	ND				
11	+	0	ND	NS[2]			-	ND				
12	+	0	ND	+	0	ND	-	ND				
13	+	0	ND	+	80	+	-	ND				
14	+	0	ND	+	2	+	-	ND				
15	+	48	-	+	100	-	-	ND				
16	+	0	ND	+	0	ND	-	ND				
17	+	0	ND	+	16	+	-	ND				
18	+	0	ND	+	0	ND	-	ND				
19	+	0	ND	+	0	ND	-	ND				

[1] Not determined: in every case of ND, the samples were negative
[2] No sample taken
A=*Streptococcus salivarius* detected
B=percentage of *Streptococcus salivarius* that have strain K12-like phenotype, as demonstrated by the inhibition of indicators I1 and I3
C=result of testing representative bacteriocin-producing isolates for genomic fingerprints typical of strain K12

indigenous SalA-producing *S. salivarius* [10]. Only one of the nasopharyngeal samples was culture-positive for strain K12. On the other hand, 3 of the 7 adenoid samples were strain K12 culture-positive.

The proportion of children newly colonised with K12-like *S. salivarius* following the taking of the powdered formulation was 6 of 18 (33%). This is substantially lower than the colonisation proportions typically achieved with use of the BLIS K12 Throat Guard™ commercial product (ca. 80%). The lower frequency obtained in the present study may, in part, be due to a reduced oral cavity exposure time for cells delivered as powdered preparations when compared to the use of lozenges. Since strain K12 is amoxicillin-sensitive, the levels of colonisation achieved in the days immediately following the amoxicillin pre-dosing would be anticipated to be relatively low. Another possible cause for the low levels of colonisation could be failure of the pre-treatment antibiotics regime to adequately reduce the levels of the indigenous *S. salivarius*. Many of the pre-colonisation *S. salivarius* recovered from the oral cavities of subjects enrolled in this study exhibited some degree of resistance to amoxicillin (results not shown). These relatively high levels of amoxicillin-resistant *S. salivarius* may be a reflection of the typical treatment histories of these subjects. All of the recruited children had experienced multiple episodes of otitis media, and these infections are often treated with amoxicillin. Although it is clear that further optimisation of the dosing protocol is still required, it appears that colonisation of the oral and adenoid tissues can be achieved in young children using a powdered probiotic formulation. The application of probiotic preparations to achieve implantation of commensal bacteria that are able to target and preclude infection by specific pathogens has considerable appeal as a cost-effective strategy to reduce the occurrence of upper respiratory tract infections in children

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