Supplementary Materials

Heavier salivary colonisation by mutans streptococci and lactobacilli without excess caries in children with congenital heart disease: a case-control study

Supplementary Figures

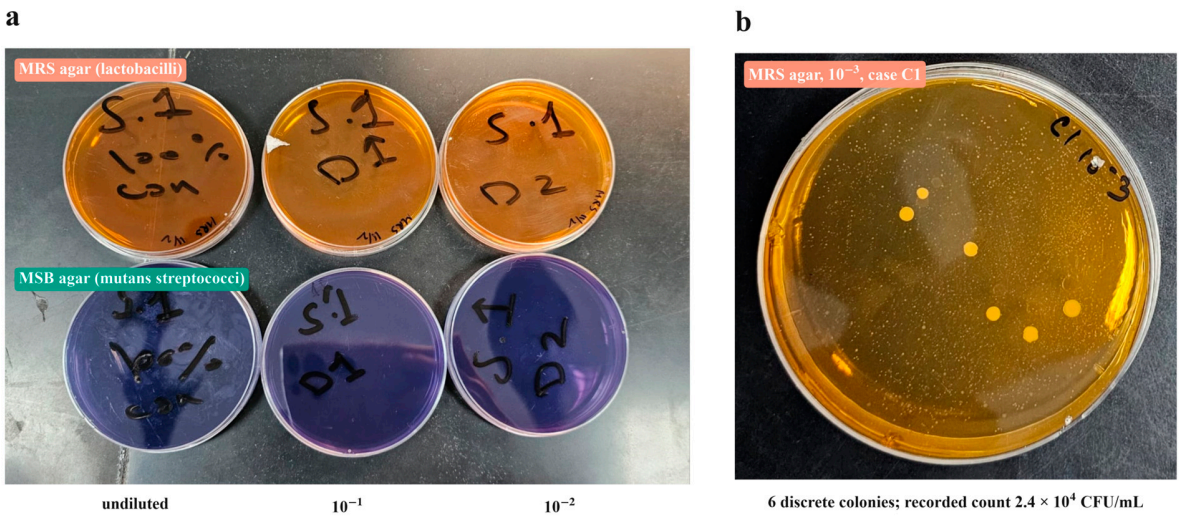


Figure S1. Representative culture plates.(A) Six plates from one saliva sample: de Man–Rogosa–Sharpe (MRS) agar for lactobacilli (top row) and Mitis Salivarius Bacitracin (MSB) agar for mutans streptococci (bottom row), plated undiluted and at 10^{-1} and 10^{-2} dilutions. (B) MRS agar plate from case C1 at the 10^{-3} dilution used for enumeration, showing six discrete, smooth, convex colonies recorded as presumptive lactobacilli; the recorded count for this sample was 2.4×10^4 CFU/mL. Photographs were taken during the study and are reproduced from the laboratory records without processing other than cropping.

Supplementary Tables

Table S1. Sensitivity analyses of the primary outcomes.

Analysis set	Outcome	CHD	Controls	Effect (95% CI)	P
Full analysis set	Mutans streptococci detected	16/30	3/26	7.64 (2.03–28.78)	0.002
Full analysis set	Lactobacilli detected	22/30	12/26	3.07 (1.03–9.16)	0.055

Full analysis set	Both detected	14/30	1/26	14.94 (2.49–89.61)	<0.001
Full analysis set	Lactobacilli log10 CFU (positives)	5.30 (n=22)	3.34 (n=12)	Δ 1.96	<0.001
Full analysis set	Mutans streptococci log10 CFU (positives)	5.57 (n=16)	4.50 (n=3)	Δ 1.07	0.074
Full analysis set	dmft	6.77 (n=30)	6.88 (n=26)	HL 0.0	0.862
Age 3–10 years only	Mutans streptococci detected	15/26	3/26	9.05 (2.33–35.17)	0.001
Age 3–10 years only	Lactobacilli detected	19/26	12/26	3.02 (0.97–9.36)	0.089
Age 3–10 years only	Both detected	13/26	1/26	17.00 (2.78–104.01)	<0.001
Age 3–10 years only	Lactobacilli log10 CFU (positives)	5.10 (n=19)	3.34 (n=12)	Δ 1.76	<0.001
Age 3–10 years only	Mutans streptococci log10 CFU (positives)	5.43 (n=15)	4.50 (n=3)	Δ 0.93	0.112
Age 3–10 years only	dmft	7.15 (n=26)	6.88 (n=26)	HL 0.0	0.832
No chronic medication	Mutans streptococci detected	15/26	3/26	9.05 (2.33–35.17)	0.001
No chronic medication	Lactobacilli detected	20/26	12/26	3.66 (1.14–11.70)	0.045
No chronic medication	Both detected	13/26	1/26	17.00 (2.78–104.01)	<0.001
No chronic medication	Lactobacilli log10 CFU (positives)	5.30 (n=20)	3.34 (n=12)	Δ 1.96	<0.001
No chronic medication	Mutans streptococci log10 CFU (positives)	5.65 (n=15)	4.50 (n=3)	Δ 1.15	0.062
No chronic medication	dmft	7.12 (n=26)	6.88 (n=26)	HL 0.0	0.883
dmft arithmetic verified only	Mutans streptococci detected	16/29	3/24	7.51 (1.97–28.65)	0.002
dmft arithmetic verified only	Lactobacilli detected	22/29	10/24	4.14 (1.32–13.03)	0.023
dmft arithmetic verified only	Both detected	14/29	1/24	14.66 (2.42–88.60)	<0.001
dmft arithmetic verified only	Lactobacilli log10 CFU (positives)	5.30 (n=22)	3.35 (n=10)	Δ 1.95	<0.001
dmft arithmetic verified only	Mutans streptococci log10 CFU (positives)	5.57 (n=16)	4.50 (n=3)	Δ 1.07	0.074
dmft arithmetic verified only	dmft	6.83 (n=29)	6.67 (n=24)	HL 0.0	0.928
First dental visit only	Mutans streptococci detected	14/26	3/26	7.79 (2.01–30.16)	0.003
First dental visit only	Lactobacilli detected	18/26	12/26	2.52 (0.83–7.66)	0.160
First dental visit only	Both detected	12/26	1/26	14.66 (2.39–89.74)	<0.001
First dental visit only	Lactobacilli log10 CFU (positives)	5.30 (n=18)	3.34 (n=12)	Δ 1.96	<0.001
First dental visit only	Mutans streptococci log10 CFU (positives)	5.43 (n=14)	4.50 (n=3)	Δ 0.93	0.126
First dental visit only	dmft	6.96 (n=26)	6.88 (n=26)	HL 0.0	1.000

Table S2. Records flagged at data verification.

id	group	age	dmft_raw	dmft_recorded	dmft	age_eligible	dmft_mismatch
C15	CHD	11.0	D5/M/F/T	5.0	5	0	0
C19	CHD	7.5	D2 / M1 / F2 / T5	10.0	5	1	1
C21	CHD	1.0	3D/M/F/T	3.0	3	0	0
C28	CHD	0.0	D1 /F4 / T	5.0	5	0	0
C29	CHD	0.0	D1 / M / F3 / T	4.0	4	0	0
H22	Healthy	7.0	D6/M0/F0/T0	16.0	6	1	1
H26	Healthy	7.0	D13/M/F/T	0.0	13	1	1

Table S3. Relationship between colonisation and caries experience.

Comparison	Group	n	Estimate	P
Mutans streptococci log10 CFU vs dmft	All	19	0.13	0.603
Mutans streptococci log10 CFU vs dmft	CHD	16	0.06	0.839
Lactobacilli log10 CFU vs dmft	All	34	-0.16	0.362
Lactobacilli log10 CFU vs dmft	CHD	22	-0.01	0.958
Lactobacilli log10 CFU vs dmft	Healthy	12	-0.09	0.771
Mutans streptococci (all, ½ LOD) vs dmft	All	56	0.01	0.961
Mutans streptococci (all, ½ LOD) vs dmft	CHD	30	0.13	0.506
Mutans streptococci (all, ½ LOD) vs dmft	Healthy	26	-0.16	0.443
Lactobacilli (all, ½ LOD) vs dmft	All	56	-0.02	0.860
Lactobacilli (all, ½ LOD) vs dmft	CHD	30	-0.09	0.641
Lactobacilli (all, ½ LOD) vs dmft	Healthy	26	0.20	0.323
dmft by mutans streptococci status	All	19+ / 37−	median 6.0 vs 6.0	0.951
dmft by mutans streptococci status	CHD	16+ / 14−	median 6.0 vs 5.0	0.599
dmft by mutans streptococci status	Healthy	3+ / 23−	median 5.0 vs 7.0	0.468
dmft by lactobacilli status	All	34+ / 22−	median 6.0 vs 5.5	0.768
dmft by lactobacilli status	CHD	22+ / 8−	median 5.0 vs 6.5	0.569
dmft by lactobacilli status	Healthy	12+ / 14−	median 8.5 vs 5.5	0.277

Table S4. Analytic power and sample-size considerations.

Outcome	Observed / assumed difference	Effect size	n per group for 80% power	Achieved power at current n
Mutans streptococci detection	53% vs 12%	h = 0.94	18	0.94
Lactobacilli detection	73% vs 46%	h = 0.56	50	0.56
Lactobacilli log10 CFU (positives)	Δ 1.96 log10	g = 1.69	7	1.00

dmft	hypothetical d = 0.3	d = 0.3	176	0.20
dmft	hypothetical d = 0.5	d = 0.5	64	0.45

Table S5. Detection and caries experience by age band.

Age band	Group	n	MS+	LB+	mean dmft
<3	CHD	3	0	2	4.0
3-5	CHD	9	6	8	5.4
3-5	Healthy	6	0	3	9.0
6-8	CHD	12	7	7	7.8
6-8	Healthy	13	2	7	7.1
9+	CHD	6	3	5	8.2
9+	Healthy	7	1	2	4.7

Table S6. Monte Carlo power (proportion of 2,000 simulated studies with P < 0.05) by children per group, for the observed effects and for effects half as large.

n_per_group	ms_detect_observed	ms_detect_half_effect	lb_detect_observed	lb_detect_half_effect	lb_load_observed	lb_load_half_effect	dmft_d05
10	0.312	0.064	0.11	0.032	0.692	0.236	0.186
15	0.562	0.127	0.199	0.061	0.882	0.362	0.261
20	0.77	0.222	0.321	0.092	0.958	0.452	0.342
26	0.883	0.311	0.425	0.118	0.991	0.57	0.418
30	0.928	0.359	0.485	0.121	0.998	0.646	0.474
40	0.984	0.472	0.644	0.172	1.0	0.759	0.603
50	0.994	0.606	0.739	0.224	1.0	0.86	0.67
60	0.999	0.669	0.832	0.263	1.0	0.908	0.766
80	1.0	0.81	0.926	0.376	1.0	0.97	0.876
100	1.0	0.91	0.972	0.47	1.0	0.994	0.94

Supplementary File

File S1. Blank parental informed consent form, the English version of the form approved by the Research Ethics Committee of the Qassim Health Cluster under approval H-04-Q-001. It carries no participant name, signature or date, and is supplied as a separate file with this submission.