*microorganisms*

MDPI

*Article*

# Heavier Salivary Colonisation by Mutans streptococci and Lactobacilli Without Excess Caries in Children with Congenital Heart Disease: A Case-Control Study

**Shahad S. Alkhuwaiter [1,*], Taif S. Alanazi [2], Murad Alrashdi [1], Sanaa Al-Haj Ali [1], Faraj Alotaiby [3], Ahmad Almatroudi [4], Monir Uddin Ahmed [4] and Khaled S. Allemailem [4,*]**

[1] Department of Orthodontic and Pediatric Dentistry, College of Dentistry, Qassim University, Buraydah 51452, Saudi Arabia; mu.alrashidi@qu.edu.sa (M.A.); s.alhajali@qu.edu.sa (S.A.-H.A.)
[2] College of Dentistry, Prince Sattam Bin Abdulaziz University, Al-Kharj 16245, Saudi Arabia; teafsaleanzi@outlook.sa
[3] Department of Oral and Maxillofacial Diagnostic Sciences, College of Dentistry, Qassim University, Buraydah 51452, Saudi Arabia; f.alotaiby@qu.edu.sa
[4] Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah 51452, Saudi Arabia; aamtrody@qu.edu.sa (A.A.); mm.ahmed@qu.edu.sa (M.U.A.)
* Correspondence: sh.alkhuwaiter@qu.edu.sa (S.S.A.); k.allemailem@qu.edu.sa (K.S.A.)

**Abstract**

Oral streptococci are the leading cause of infective endocarditis in children with congenital heart disease (CHD), and whether these children carry a heavier cariogenic bacterial burden than healthy children, independently of caries, is unresolved. Unstimulated saliva from 30 children with CHD and 26 healthy children aged 3–10 years was cultured on selective agars; dmft and oral-health behaviour were recorded; and counts were analysed on the logarithmic scale with exact tests, Firth penalised and negative-binomial regression, left-censored (Tobit) models, permutation tests, E-values, and Monte Carlo simulation. Mutans streptococci were cultured from 53.3% of children with CHD and 11.5% of controls (Firth age-adjusted odds ratio 8.9, 95% CI 2.5–40.3) and lactobacilli from 73.3% and 46.2% (3.0, 1.0–9.4), with a 1.96 log10 CFU/mL higher lactobacillus load among carriers ($p < 0.001$). Caries experience was identical (dmft 6.77 versus 6.88), but children with CHD more often had treated teeth (53.3% versus 11.5%). The associations survived adjustment, penalisation, and censoring; explaining them away would require an unmeasured confounder with risk ratios of 3–10. Simulation showed that the observable odds ratio rises steeply with the culture detection floor, reconciling conflicting published results. In this sample, caries status did not indicate cariogenic burden. The finding is preliminary and requires confirmation with confirmed organism identification and larger groups.

**Keywords:** congenital heart disease; *Streptococcus mutans*; mutans streptococci; lactobacilli; dental caries; saliva; infective endocarditis; children; Firth regression; Tobit regression

Check for updates

Academic Editor: Hidenobu Senpuku

Received: 9 November 2026
Revised: 19 November 2026
Accepted: 25 November 2026
Published: 27 November 2026

**Copyright:** © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

## 1. Introduction

Congenital heart disease (CHD) is the most common major birth defect. The Global Burden of Disease Study 2023 estimates that 4.1 million children under five years of age were living with a congenital heart anomaly in 2023, a prevalence of 639 per 100,000 (95% uncertainty interval 549–731) [1]. Advances in surgical and catheter-based repair mean that most of these children now survive into adulthood, and their long-term care increasingly concerns comorbidities that were once secondary, among them oral disease [2–5]. Dental

caries in the primary dentition remains one of the most prevalent conditions of childhood worldwide, affecting an estimated 38.6% of children aged 5–9 years in 2023, with little change since 1990 [1,6–8].

The oral cavity matters in CHD for a specific reason. Viridans group streptococci and other oral bacteria enter the bloodstream during chewing, tooth-brushing, and dental treatment, and in children with structural heart lesions, prosthetic material, or previous endocarditis, these bacteraemias can seed the endocardium [9–11]. Current guidance from the American Heart Association places maintenance of oral health above antibiotic prophylaxis as the principal strategy for preventing viridans streptococcal endocarditis and reserves prophylaxis for the highest-risk cardiac conditions [9]. The strength of that recommendation depends on knowing whether children with CHD actually carry a heavier burden of oral bacteria capable of causing both caries and bacteraemia and whether that burden is captured by clinical examination.

Dental caries is a biofilm-mediated, sugar-driven disease in which acidogenic and aciduric bacteria shift the balance between demineralisation and remineralisation of enamel [7,12,13]. Mutans streptococci, chiefly *Streptococcus mutans* and *Streptococcus sobrinus*, initiate acid production and adhere to enamel through glucan synthesis, and *S. mutans* combines acid production, acid tolerance, and extracellular polysaccharide synthesis in a single organism to an extent that few other oral species match [14]; lactobacilli are markers of an established low-pH niche and are associated with lesion progression rather than initiation [12,15–17]. Both groups are readily cultured from saliva on selective media, and salivary counts above about $10^5$ colony-forming units (CFU) per millilitre for mutans streptococci or $10^4$ CFU/mL for lactobacilli have long been used as thresholds of elevated caries risk [18]. Several features of life with CHD could raise these counts: frequent feeding to meet energy demands, sweetened liquid medicines, drugs that reduce salivary flow, repeated antibiotic courses, and hospitalisation that interrupts daily oral care [2,5,19–22].

Whether these exposures translate into measurably heavier colonisation is unclear. A narrative review of 14 controlled studies found that only half reported a difference in colonisation, and the direction varied [19]. Studies using selective culture in Iran, Sweden, and India have reported higher salivary mutans streptococci in CHD [23–25], whereas culture-based studies in the United Kingdom, Turkey, and Brazil found no difference or a lower count in cardiac children [26–28]. Quantitative PCR (qPCR) in Sudan detected *S. mutans* in almost every child regardless of cardiac status, with a modest difference in load [29], and a Turkish qPCR study found no difference in either detection or counts [30]. For lactobacilli, the data are sparser and, where available, point in opposite directions [26–28]. Culture-independent profiling of carious lesions in children with CHD has begun [31], but comparative data on the organisms themselves remain confined to small culture studies. A 2021 review found the evidence of excess caries in CHD inconsistent [32], and a 2024 meta-analysis pooled dmft across studies and found no significant difference (mean difference 1.39, 95% CI −1.05 to 3.83) [33].

Two further issues limit interpretation of the existing studies. Most report bacterial load as arithmetic means of CFU counts, which are dominated by a few heavily colonised children, whereas microbial counts are approximately normal only on the logarithmic scale; and none has examined how the handling of culture-negative samples, the sparseness of positive controls, or the detection floor of the culture method itself shapes the comparison. Substituting a fixed value for non-detects biases both means and variances [34], maximum-likelihood logistic regression becomes unstable when a cell count approaches zero [35,36], and a culture that scores presence above a fixed dilution answers a different question from a molecular assay that scores presence at all.

This study therefore compared salivary mutans streptococci and lactobacilli, quantified on the logarithmic scale, with caries experience and its decayed, missing, and filled components in children with CHD and healthy children, adjusting for age and oral-health behaviour. The analysis was designed to test whether any microbiological difference survives the statistical problems that small culture studies face: sparse positive counts, left-censored loads, unmeasured confounding, and the dependence of detection on the culture floor.

## 2. Materials and Methods

### *2.1. Study Design and Participants*

A case-control study was conducted in a tertiary paediatric cardiology centre and a university paediatric dental clinic in Qassim Province, Saudi Arabia. Cases were children with a cardiologist-confirmed structural congenital heart defect under regular follow-up; controls were children classified as American Society of Anesthesiologists physical status I attending their first dental visit at the university clinic. The protocol specified an age range of 3–10 years, Saudi nationality, residence in the same administrative region, and Arabic or English as the family language; children with a diagnosed syndrome, physical or intellectual disability, or whose parent or guardian declined participation were excluded. Recruitment was by convenience sampling. The protocol was approved by the Research Ethics Committee of the Qassim Health Cluster, Saudi Arabia (protocol code H-04-Q-001, date of approval 4 October 2023), and written informed consent was obtained from a parent or guardian of every child.

Thirty cases and 30 controls were enrolled. Saliva was not obtained from four controls, who were excluded from all analyses; the analysis set therefore comprised 30 children with CHD and 26 healthy children. Verification of the case report forms against the dataset identified four cases outside the protocol age range (0, 0, 1, and 11 years) and three records in which the recorded dmft total did not equal the sum of its components (one case, two controls). All children were retained in the primary analysis with the component sum used as the dmft value, and the effect of excluding these records was examined in sensitivity analyses.

The original sample size was derived from a single-proportion precision formula (expected prevalence 10%, precision 5%, 95% confidence), which does not address a two-group comparison; the precision the study actually achieved is therefore reported with the results, as a confidence interval around every effect estimate.

### *2.2. Clinical Examination and Questionnaire*

Caries experience in the primary dentition was recorded as the number of decayed (d), missing because of caries (m), and filled (f) teeth according to World Health Organization criteria, with lesions recorded at the cavitated threshold [37]. The dmft index was computed as d + m + f. Two derived measures were used: the untreated share, d/dmft among children with dmft > 0, and treatment experience, defined as at least one missing or filled tooth. An interviewer-administered questionnaire recorded age, nationality, region of residence and family language, cardiac diagnosis, and current medication (cases), dental history (first visit or prior treatment), regular dental recall, tooth-brushing frequency, parental help with brushing, flossing, mouthwash use, previous professional topical fluoride, parental attitude to fluoride, and the daily frequency of free-sugar exposure, the behaviours most consistently associated with caries status in children [38,39]. All clinical examinations were performed by a single calibrated examiner (a paediatric dentist) under standardised conditions using a plane mouth mirror and a WHO periodontal probe (a ball-ended probe of the specification set out in the World Health Organization basic methods, with a 0.5 mm ball tip) after drying the teeth with gauze. Before the study, the examiner was calibrated against an

experienced paediatric dentist on 10 children who were not part of the sample, and 10% of the participants were re-examined one week later to assess intra-examiner reliability (Cohen's kappa = 0.9). The type of cardiac lesion, cyanosis, and surgical status were not recorded for individual cases.

Nationality, region of residence and family language were recorded to confirm eligibility and were not used in the analysis. Free-text questionnaire entries were coded before analysis using fixed rules. Tooth-brushing was coded as less than once daily, once daily, or at least twice daily; free-sugar exposure as fewer than once, once, twice, or three or more times per day. Chronic medication was coded as present when any regular cardiac medication was recorded (aspirin, warfarin, captopril, furosemide, or lisinopril in this cohort).

### *2.3. Saliva Collection and Culture*

Two millilitres of unstimulated whole saliva were collected from each child. Samples were serially diluted in saline and plated undiluted and at $10^{-1}$ to $10^{-3}$ on Mitis Salivarius Bacitracin agar (Merck KGaA, Darmstadt, Germany), incubated at 37 °C under anaerobic conditions for 24–48 h, and on de Man–Rogosa–Sharpe agar (Merck KGaA, Darmstadt, Germany), incubated at 37 °C aerobically for 24–48 h. Colonies with the raised, convex, frosted-glass morphology characteristic of mutans streptococci on Mitis Salivarius Bacitracin agar and white colonies on de Man–Rogosa–Sharpe agar were counted and recorded as presumptive mutans streptococci and lactobacilli, respectively. Identification was morphological only; no biochemical, mass-spectrometric, or molecular confirmation was performed, and isolates were not stored. The organisms are therefore reported throughout as mutans streptococci and lactobacilli, not as *S. mutans* and *Lactobacillus acidophilus*. Colonies were counted on the dilution that gave discrete, countable growth and converted to CFU/mL of saliva from the plated volume and dilution factor; for the enumeration plate of case C1, six colonies at $10^{-3}$ corresponded to a recorded $2.4 \times 10^4$ CFU/mL, consistent with a 0.25 mL inoculum. The lowest count recorded in the study, $1 \times 10^2$ CFU/mL, was used as an operational value for the analysis and not as a validated limit of detection. It is the smallest count the dilution series and plating volume could return, not a threshold established by spiking a known inoculum, and it applies to selective culture alone: molecular methods detect smaller numbers of cells, including cells that do not grow on these media, so this value is not equivalent to a molecular detection floor. Each dilution was plated in duplicate, and the mean of the two counts was used. Anaerobic incubation was carried out in an anaerobic jar with a gas-generating sachet (AnaeroGen, Oxoid, Basingstoke, UK) and an anaerobic indicator strip.

### *2.4. Primary Statistical Analysis*

Bacterial counts were log10-transformed. Two complementary analyses of load were specified: among culture-positive children only, and among all children with non-detects set to half the detection floor ($5 \times 10^1$ CFU/mL). High-count carriage was defined as $\geq 10^5$ CFU/mL for either organism, with $\geq 10^4$ CFU/mL for lactobacilli as an additional threshold for comparability with published studies. Group differences in proportions were tested with Fisher's exact test and expressed as odds ratios (OR) estimated by conditional maximum likelihood with exact 95% confidence intervals (CI) and as risk differences. The exact interval is the interval that corresponds to Fisher's exact test, so the interval and the *p* value reported for the same comparison are derived from one procedure. It is conservative by construction, and the penalised and maximum-likelihood odds ratios reported with the regression results carry profile-likelihood intervals, which may therefore differ from it for a comparison lying close to the conventional threshold. Continuous outcomes were compared with the Mann–Whitney U test and Welch's *t*-test, with the Hodges–Lehmann

shift and bootstrap 95% CI for the median difference, Cliff's δ, and Hedges g. Logistic regression estimated the association of CHD status with each microbiological outcome, crude, adjusted for age, and adjusted for age, daily tooth-brushing, and free-sugar exposure three or more times per day. Linear regression with the same covariates estimated the adjusted difference in log10 load. Caries counts were modelled with negative-binomial regression (dispersion estimated by profile likelihood) adjusted for age and, in further models, for organism status and behaviour. Spearman correlation related log10 load to dmft. Sensitivity analyses restricted the sample to children aged 3–10 years, to children without chronic medication, to records with verified dmft arithmetic, and to children at their first dental visit. Inference rests on the effect estimates and their confidence intervals. Power computed after the event at the observed effect size carries no information beyond the interval itself and is not used to interpret any result here. The several models were specified before the analysis, and all of them are reported, because with 30 and 26 children no single model can carry the conclusion: each makes its own assumption about sparse cells, censored counts, or the shape of the distribution, and a finding is persuasive only if it survives all of them ($\alpha = 0.05$, two-sided, for every test reported).

### *2.5. Robustness Analyses and Simulation*

Four problems that affect small culture-based comparisons were addressed with analyses specified before the robustness results were examined. First, because only three controls were culture-positive for mutans streptococci and no control carried lactobacilli at $\geq 10^5$ CFU/mL, every logistic model was refitted with Firth's penalised likelihood [35], which removes the small-sample bias of maximum likelihood and yields finite estimates under separation [36]; 95% CIs and $p$ values were obtained from the profile penalised likelihood. Second, because substitution of a fixed value for non-detects distorts both the mean and the variance of a censored variable [34], the all-children load models were refitted as left-censored normal (Tobit) regressions [40] in which a non-detect contributes the probability of a value at or below the detection floor instead of an imputed value; the model was applied only where at least 25% of each group was culture-positive, since the censored-normal distribution is not identifiable when nearly all values in one group are censored. Third, E-values [41] quantified the minimum strength of association on the risk-ratio scale that an unmeasured confounder would need with both CHD status and the outcome to explain away each fully adjusted association and its confidence limit; odds ratios were converted to approximate risk ratios by their square root because the outcomes were common, and standardised load differences by $\exp(0.91 \times d)$. Fourth, the main contrasts were re-tested with permutation tests (20,000 random relabellings of group membership), and the standardised load differences were given bootstrap percentile CIs (10,000 resamples).

Two Monte Carlo simulations were then run. The first asked how the detection floor of the culture method shapes the observable odds ratio: a censored-normal distribution of log10 load was fitted to each group, parameters were drawn 4000 times from their asymptotic sampling distributions, and for detection floors from $10^{0.5}$ to $10^6$ CFU/mL, the expected detection probability in each group and the resulting odds ratio were computed, giving a point curve with a 95% simulation band. The second estimated the power of future studies: for group sizes from 10 to 100, 2000 datasets per size were simulated from the observed detection proportions and log10 load distributions and from scenarios with the effect halved on the log-odds or mean-difference scale, and they were analysed with Fisher's exact test or Welch's $t$-test at $\alpha = 0.05$. All analyses used Python 3.13 with NumPy 2.5, pandas 3.1, SciPy 1.18, and statsmodels 0.14 and Matplotlib 3.11 with a fixed random seed; the analysis code and the cleaned dataset are provided as Supplementary Material.

### *2.6. Population Burden Estimates*

Prevalence of caries of deciduous teeth (ages 1–4 and 5–9 years) and of congenital heart anomalies (under 5 years) for 2023, by Global Burden of Disease super-region, Socio-demographic Index quintile and country, with 1990–2023 time series, were obtained from the Institute for Health Metrics and Evaluation Global Burden of Disease Study 2023 [1]. These estimates were used to describe the population setting of the study and were not combined with the study data.

## 3. Results

### *3.1. The Population Burden of Caries and Congenital Heart Disease*

Caries of deciduous teeth affected 40.4% (95% UI 32.0–47.9) of children aged 1–4 years and 38.6% (30.6–47.4) of children aged 5–9 years worldwide in 2023, with super-region prevalence at 5–9 years ranging from 35.0% in high-income countries to 42.6% in Central Europe, Eastern Europe, and Central Asia (Figure 1a). Prevalence in this age band has moved by only a few percentage points since 1990 in every super-region (Figure 1b). Congenital heart anomalies were present in 639 per 100,000 children under five years globally, from 555 per 100,000 in Southeast Asia, East Asia, and Oceania to 891 per 100,000 in Central Europe, Eastern Europe, and Central Asia (Figure 1c). Across 204 countries, the two burdens were weakly and inversely correlated (Spearman $\rho = -0.16$, $p = 0.02$; Figure 1d). The study setting had one of the higher estimated deciduous caries prevalences at 5–9 years (57.9%, 47.6–67.9) and a CHD prevalence close to the global value (558 per 100,000), so the cohort was drawn from a population in which caries is near-universal and CHD is as common as anywhere.

### *3.2. Participants*

The analysis set comprised 30 children with CHD and 26 healthy controls (Table 1). Mean age was 6.1 ± 2.8 years in the CHD group and 6.9 ± 1.7 years in controls ($p = 0.36$). Four children with CHD (13.3%) were on regular cardiac medication and five (16.7%) attended regular dental recall; all controls and 26 of 30 cases were at their first dental visit. Children with CHD more often received parental help with brushing (30.0% versus 7.7%, $p = 0.047$) and were less often exposed to free sugars three or more times a day (20.0% versus 42.3%, $p = 0.087$); daily tooth-brushing was reported for 80.0% and 57.7%, respectively ($p = 0.087$). Flossing, mouthwash use, previous professional fluoride, and parental opposition to fluoride were rare in both groups.

**Table 1.** Characteristics of the participants.

| Characteristic | CHD ($n = 30$) | Healthy Controls ($n = 26$) | $p$ | Test |
|---|---|---|---|---|
| Age, years, mean ± SD | 6.1 ± 2.8 | 6.9 ± 1.7 | 0.362 | Mann–Whitney U |
| Age, years, median (IQR) | 6.0 (5.0–8.0) | 7.0 (6.0–8.8) | | |
| Age 3–10 years (protocol range) | 26 (86.7) | 26 (100.0) | 0.115 | Fisher exact |
| First dental visit | 26 (86.7) | 26 (100.0) | 0.115 | Fisher exact |
| Regular dental recall | 5 (16.7) | 0 (0.0) | 0.055 | Fisher exact |
| Chronic medication | 4 (13.3) | 0 (0.0) | 0.115 | Fisher exact |
| Brushes at least once daily | 24 (80.0) | 15 (57.7) | 0.087 | Fisher exact |
| Parental help with brushing | 9 (30.0) | 2 (7.7) | 0.047 | Fisher exact |
| Flossing | 1 (3.3) | 0 (0.0) | 1.000 | Fisher exact |
| Mouthwash use | 1 (3.3) | 0 (0.0) | 1.000 | Fisher exact |
| Previous topical fluoride | 1 (3.3) | 0 (0.0) | 1.000 | Fisher exact |

**Table 1.** *Cont.*

| Characteristic | CHD ($n$ = 30) | Healthy Controls ($n$ = 26) | $p$ | Test |
|---|---|---|---|---|
| Parent against fluoride | 2 (6.7) | 1 (3.8) | 1.000 | Fisher exact |
| Free-sugar exposure ≥ 3 times/day | 6 (20.0) | 11 (42.3) | 0.087 | Fisher exact |
| Brushing frequency, categories | 6/18/6 | 11/12/3 | 0.182 | Chi-square |
| Sugar exposure, categories | 6/7/11/6 | 0/6/9/11 | 0.058 | Chi-square |

Values are $n$ (%) unless stated. Brushing categories: <1/day, 1/day, ≥2/day. Sugar categories: <1, 1, 2, ≥3 exposures/day. $p$ values from Fisher's exact test, Mann–Whitney U test or chi-square test as indicated.

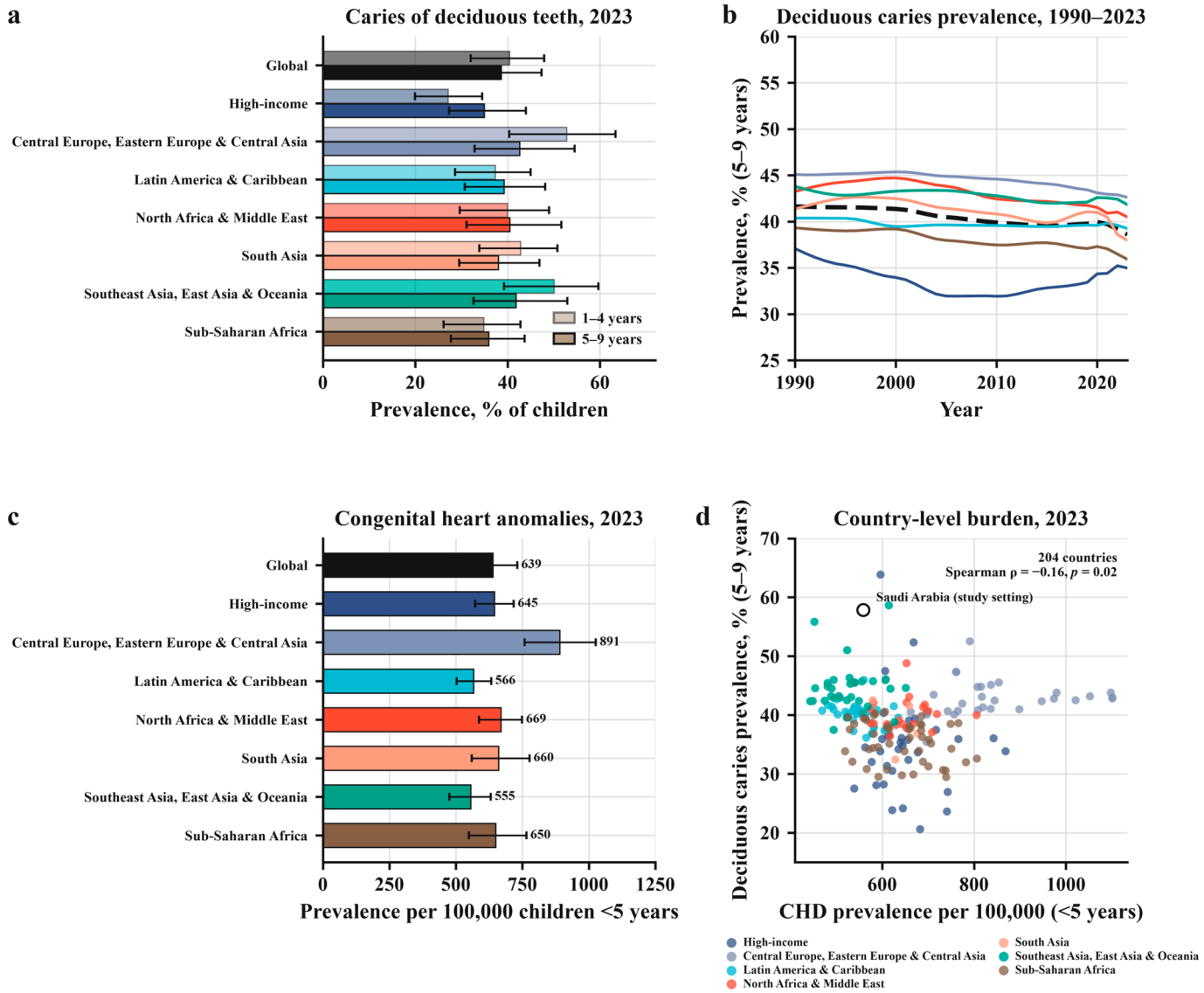



**Figure 1.** Population burden of caries of deciduous teeth and congenital heart anomalies, Global Burden of Disease Study 2023. (**a**) Prevalence of caries of deciduous teeth at ages 1–4 years (light) and 5–9 years (dark) by GBD super-region, with 95% uncertainty intervals. (**b**) Prevalence at 5–9 years, 1990–2023, by super-region (dashed black line, global). (**c**) Prevalence of congenital heart anomalies in children under five years per 100,000, with 95% uncertainty intervals and point estimates. (**d**) Country-level prevalence of deciduous caries at 5–9 years against congenital heart anomaly prevalence under five years for 204 countries, coloured by super-region; the study setting is circled and labelled. Spearman correlation across countries is shown. Colours denote GBD super-regions and are the same in (**a**–**c**); the key in (**d**) applies to all four panels. In (**b**) the dashed black line is the global estimate.

### 3.3. Caries Experience and Treatment Experience

Caries experience was high and indistinguishable between groups: dmft 6.77 ± 4.40 (median 5.0, IQR 4.2–9.5) in CHD and 6.88 ± 4.58 (median 6.5, IQR 4.0–9.8) in controls (Hodges–Lehmann difference 0.0, 95% CI −3.5 to 2.5; Cliff's δ −0.03; $p = 0.86$; Figure 2a, Table 2). Two children with CHD (6.7%) and four controls (15.4%) were caries-free ($p = 0.40$; Figure 2c). The composition of the index differed. The decayed component was numerically lower in CHD (5.10 ± 4.25 versus 6.04 ± 4.80, $p = 0.40$), whereas the missing (0.50 ± 0.94 versus 0.23 ± 1.18, $p = 0.017$) and filled (1.17 ± 2.04 versus 0.62 ± 2.08, $p = 0.015$) components were higher (Figure 2b). Among children with caries, the untreated share of the index was 0.76 ± 0.29 in CHD and 0.90 ± 0.30 in controls ($p = 0.007$; Figure 2d), and 53.3% of children with CHD had at least one missing or filled tooth compared with 11.5% of controls (OR 8.41, 95% CI 1.92–53.10, $p = 0.002$; Figure 2c), an association that persisted after adjustment for age and dmft (OR 11.38, 2.35–55.08). In negative-binomial models, dmft was unrelated to CHD status after adjustment for age (incidence rate ratio 0.99, 0.68–1.44; Figure 2e) and after further adjustment for organism status and behaviour (1.00, 0.63–1.59).

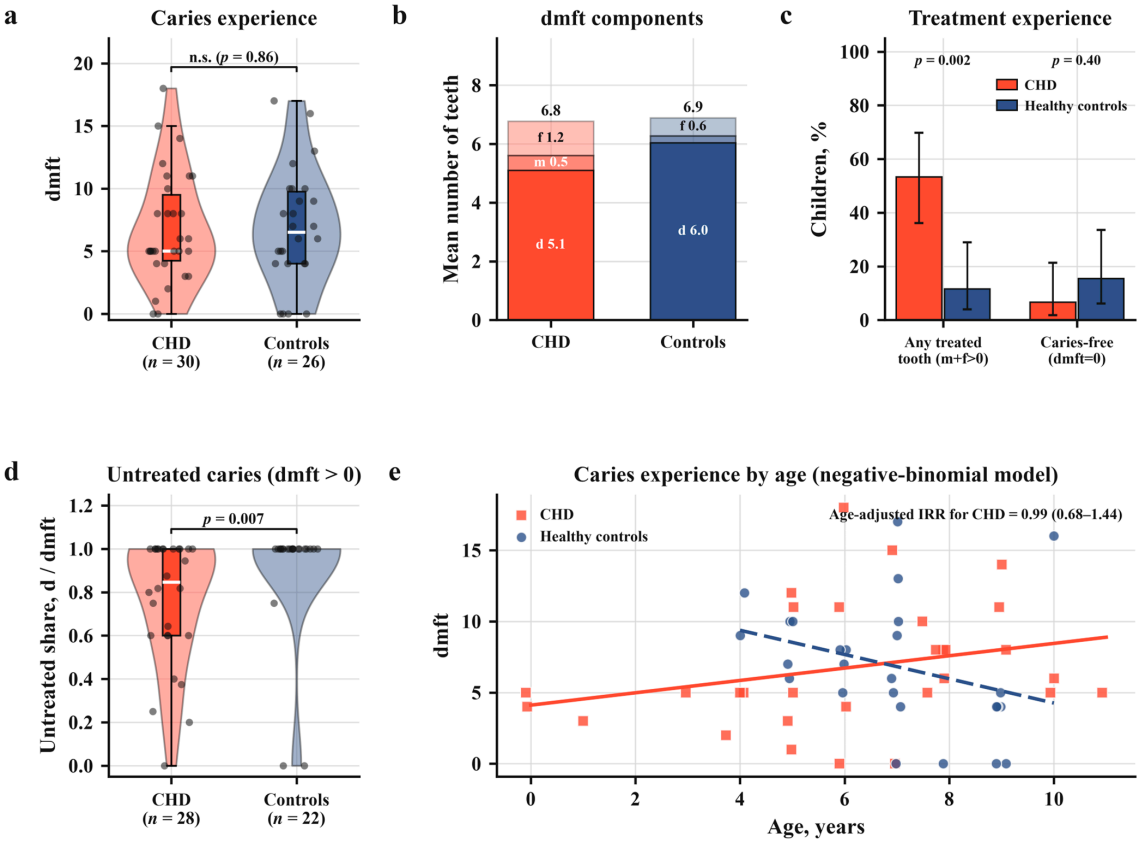



**Figure 2.** Caries experience in children with congenital heart disease (CHD) and healthy controls. (**a**) dmft by group; violins show the distribution, boxes the median and interquartile range, points individual children. (**b**) Mean decayed (d), missing (m), and filled (f) components of dmft. (**c**) Proportion of children with at least one treated (missing or filled) tooth and proportion caries-free, with Wilson 95% confidence intervals and Fisher exact $p$ values. (**d**) Untreated share of the index (d/dmft) among children with dmft > 0. (**e**) dmft against age with linear fits; the incidence rate ratio is from an age-adjusted negative-binomial model. $n = 30$ CHD, 26 controls. Red denotes children with CHD and blue healthy controls throughout; grey points are individual children. In (**b**) the colour intensity decreases from the decayed to the filled component and each component mean is printed inside the bar. In (**e**) the solid line is the fit for children with CHD and the dashed line the fit for controls.

**Table 2.** Caries experience in the primary dentition.

| Outcome | CHD Mean ± SD | CHD Median (IQR) | Control Mean ± SD | Control Median (IQR) | Difference (95% CI) | Cliff's δ | *p* |
|---|---|---|---|---|---|---|---|
| dmft | 6.77 ± 4.40 | 5.0 (4.2–9.5) | 6.88 ± 4.58 | 6.5 (4.0–9.8) | 0.0 (−3.5 to 2.5) | −0.03 | 0.862 |
| Decayed (d) | 5.10 ± 4.25 | 4.0 (2.2–6.8) | 6.04 ± 4.80 | 5.5 (3.2–8.8) | −1.0 (−4.5 to 1.5) | −0.13 | 0.404 |
| Missing (m) | 0.50 ± 0.94 | 0.0 (0.0–1.0) | 0.23 ± 1.18 | 0.0 (0.0–0.0) | 0.0 (0.0 to 0.0) | 0.25 | 0.017 |
| Filled (f) | 1.17 ± 2.04 | 0.0 (0.0–2.0) | 0.62 ± 2.08 | 0.0 (0.0–0.0) | 0.0 (0.0 to 1.5) | 0.30 | 0.015 |
| Untreated share d/dmft (dmft > 0) | 0.76 ± 0.29 | 0.8 (0.6–1.0) | 0.90 ± 0.30 | 1.0 (1.0–1.0) | −0.1 (−0.4 to 0.0) | −0.39 | 0.007 |
| Caries-free (dmft = 0), *n* (%) | 2 (6.7) | | 4 (15.4) | | OR 0.44 (0.08–2.27) | | 0.401 |
| Any treated tooth (m + f > 0), *n* (%) | 16 (53.3) | | 3 (11.5) | | OR 7.64 (2.03–28.78) | | 0.002 |

dmft, decayed, missing, and filled primary teeth (component sum). Difference is the Hodges–Lehmann median shift with bootstrap 95% CI, or the conditional maximum likelihood odds ratio with exact 95% CI for proportions. P from Mann–Whitney U or Fisher's exact test.

### *3.4. Carriage of Mutans streptococci and Lactobacilli*

Mutans streptococci were cultured from 16 of 30 children with CHD (53.3%) and 3 of 26 controls (11.5%), a risk difference of 41.8 percentage points (95% CI 20.1–63.5) and OR 8.41 (1.92–53.10; $p = 0.002$; Figure 3a, Table 3); representative culture plates are shown in Figure S1. Carriage at $\geq 10^5$ CFU/mL was present in 10 (33.3%) and 1 (3.8%), respectively (OR 12.03, 1.49–563.62; $p = 0.007$). Lactobacilli were cultured from 22 (73.3%) and 12 (46.2%) (risk difference 27.2 points, 2.3–52.0; OR 3.14, 0.92–11.46; $p = 0.055$), and at $\geq 10^5$ CFU/mL from 10 (33.3%) and none ($p < 0.001$). Both organisms were detected together in 14 children with CHD (46.7%) and one control (3.8%; OR 20.81, 2.69–960.87; $p < 0.001$), and the distribution of the number of organisms detected (none, one, or both) differed between groups ($\chi^2$ $p = 0.001$; Figure 4a). In logistic models adjusted for age, the OR for mutans streptococci was 10.53 (2.47–44.89) and for lactobacilli 3.22 (1.03–10.04); further adjustment for daily brushing and frequent sugar exposure did not attenuate either association, although the maximum-likelihood estimate for mutans streptococci became imprecise (44.7, 3.9–508; Figure 3d).

**Table 3.** Culture detection of mutans streptococci and lactobacilli in saliva.

| Outcome | CHD *n*/N (%) | Controls *n*/N (%) | OR (95% CI) | Risk Difference, % (95% CI) | P (Fisher) |
|---|---|---|---|---|---|
| Mutans streptococci detected | 16/30 (53.3) | 3/26 (11.5) | 7.64 (2.03–28.78) | 41.8 (20.1 to 63.5) | 0.002 |
| Mutans streptococci $\geq 10^5$ CFU/mL | 10/30 (33.3) | 1/26 (3.8) | 8.71 (1.43–53.04) | 29.5 (11.1 to 47.9) | 0.007 |
| Lactobacilli detected | 22/30 (73.3) | 12/26 (46.2) | 3.07 (1.03–9.16) | 27.2 (2.3 to 52.0) | 0.055 |
| Lactobacilli $\geq 10^5$ CFU/mL | 10/30 (33.3) | 0/26 (0.0) | 27.15 (1.50–490.94) | 33.3 (16.5 to 50.2) | <0.001 |
| Both organisms detected | 14/30 (46.7) | 1/26 (3.8) | 14.94 (2.49–89.61) | 42.8 (23.5 to 62.1) | <0.001 |
| Either organism detected | 24/30 (80.0) | 14/26 (53.8) | 3.25 (1.03–10.25) | 26.2 (2.2 to 50.1) | 0.048 |

OR, conditional maximum likelihood odds ratio with exact 95% CI, the interval that corresponds to Fisher's exact test; risk difference with Wald 95% CI; P from Fisher's exact test. Detection floor $10^2$ CFU/mL (the lowest count recorded, used as an operational floor and not a validated limit of detection).

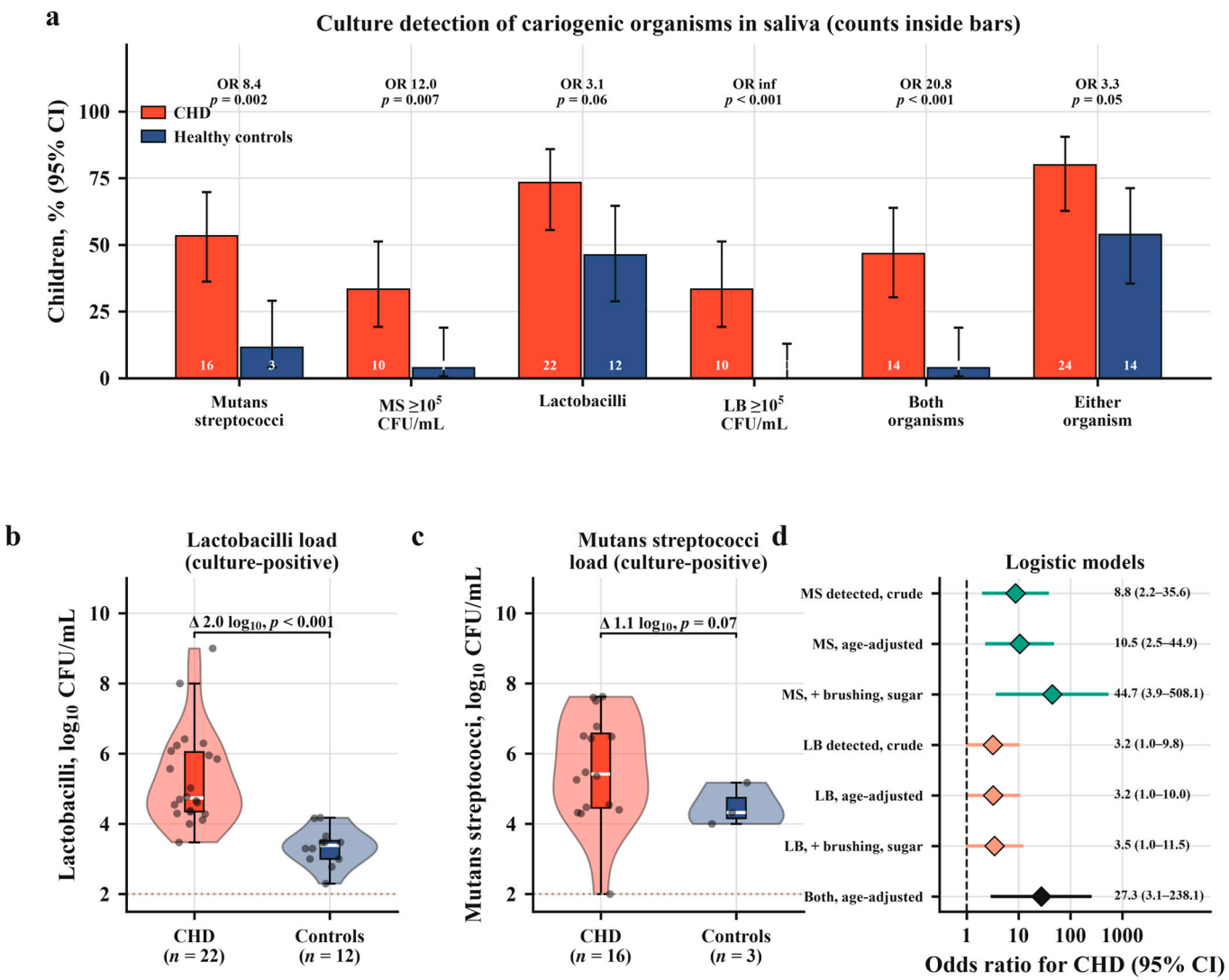



**Figure 3.** Culture detection and load of cariogenic organisms in saliva. (**a**) Percentage of children in whom mutans streptococci (MS) and lactobacilli (LB) were detected, detected at ≥$10^5$ CFU/mL, both detected, or either detected, with Wilson 95% confidence intervals; counts are given inside bars and odds ratios with Fisher exact *p* values above. (**b**,**c**) log10 CFU/mL among culture-positive children for lactobacilli and mutans streptococci; dotted line, detection floor ($10^2$ CFU/mL); brackets give the mean difference and Welch *p* value. (**d**) Odds ratios for CHD from maximum-likelihood logistic models, crude, age-adjusted, and adjusted for age, daily tooth-brushing, and free-sugar exposure ≥ 3 times/day; diamonds, point estimates; lines, 95% confidence intervals (log scale). Red denotes children with CHD and blue healthy controls; grey points are individual children. In (**d**) teal diamonds are mutans streptococci, salmon diamonds lactobacilli and the black diamond both organisms.

### *3.5. Bacterial Load*

Among culture-positive children, lactobacillus load was 5.30 ± 1.34 log10 CFU/mL in CHD ($n = 22$) and 3.34 ± 0.54 in controls ($n = 12$), a difference of 1.96 log10 units (Hedges g 1.69; Welch $p < 0.001$; Mann–Whitney $p < 0.001$), corresponding to geometric means of $2.0 \times 10^5$ and $2.2 \times 10^3$ CFU/mL (Figure 3b, Table 4). Mutans streptococci load among positives was 5.57 ± 1.54 ($n = 16$) versus 4.50 ± 0.61 ($n = 3$), a difference of 1.07 log10 units that did not reach significance with three control positives ($p = 0.07$; Figure 3c). When all children were included with non-detects at half the detection floor, both loads were higher in CHD: lactobacilli 4.34 ± 1.98 versus 2.46 ± 0.91 (g 1.18, $p < 0.001$) and mutans streptococci 3.76 ± 2.25 versus 2.02 ± 0.93 (g 0.97, Welch $p < 0.001$, Mann–Whitney $p < 0.001$). After

adjustment for age, daily brushing, and frequent sugar exposure, CHD was associated with a 1.98 log10 (1.07–2.89) higher lactobacillus load and a 2.14 log10 (1.26–3.02) higher mutans streptococci load (both $p < 0.001$; Figure 4e).

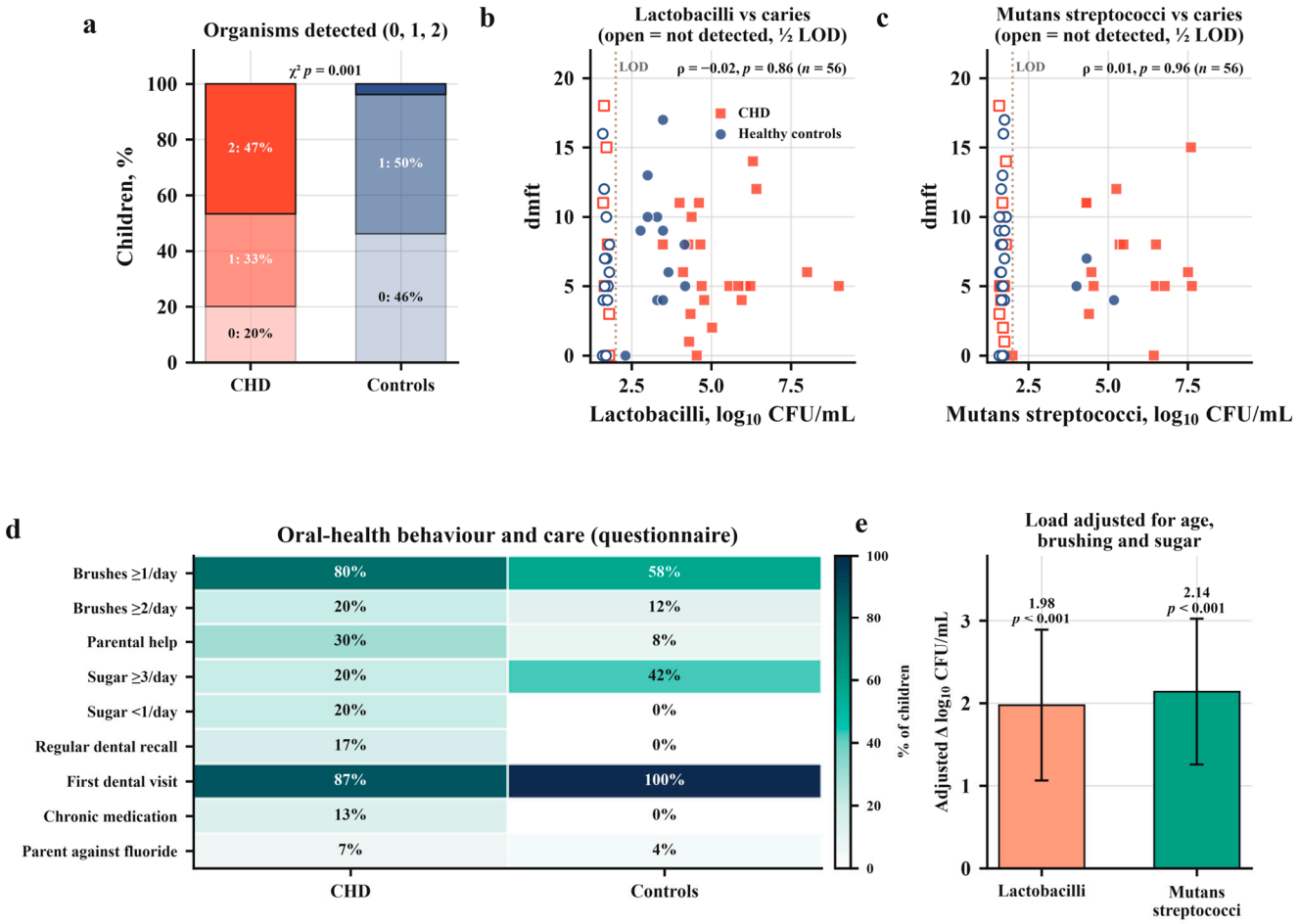



**Figure 4.** Colonisation in relation to caries experience and oral-health behaviour. (**a**) Distribution of the number of organisms detected (0, 1, or 2) by group with $\chi^2$ $p$ value. (**b**,**c**) dmft against log10 CFU/mL of lactobacilli and mutans streptococci for all children; filled symbols, culture-positive; open symbols, not detected and plotted at half the detection floor (LOD); Spearman ρ across all children. (**d**) Questionnaire-reported behaviour and care by group, percentage of children. (**e**) Adjusted difference in log10 CFU/mL (CHD minus controls) from linear models with non-detects at half the detection floor, adjusted for age, daily brushing, and frequent sugar exposure, with 95% confidence intervals. Red squares denote children with CHD and blue circles healthy controls; the dotted vertical line in (**b**,**c**) marks the detection floor (LOD). In (**d**) the colour scale gives the percentage of children. In (**e**) salmon denotes lactobacilli and teal mutans streptococci.

### *3.6. Colonisation in Relation to Caries*

Neither load correlated with dmft among culture-positive children (lactobacilli $\rho = -0.16$, $p = 0.36$, $n = 34$; mutans streptococci $\rho = 0.13$, $p = 0.60$, $n = 19$) or among all children with non-detects imputed ($\rho = -0.02$ and 0.01; Figure 4b,c; Table S3). Median dmft was the same in children with and without mutans streptococci (6.0 versus 6.0, $p = 0.95$) and with and without lactobacilli (6.0 versus 5.5, $p = 0.77$), and in the negative-binomial model, neither organism predicted dmft (incidence rate ratios 0.89 and 0.98; Table 5). The behavioural profile of the two groups is summarised in Figure 4d.

**Table 4.** Salivary bacterial load.

| Load | CHD *n* | CHD log10 CFU/mL | CHD Geometric Mean | Control *n* | Control log10 CFU/mL | Control Geometric Mean | Δ log10 | Hedges g | *p* (Welch) | *p* (MWU) |
|---|---|---|---|---|---|---|---|---|---|---|
| Mutans streptococci, culture-positive only | 16 | 5.57 ± 1.54 | $3.69 \times 10^5$ | 3 | 4.50 ± 0.61 | $3.16 \times 10^4$ | 1.07 | 0.70 | 0.074 | 0.117 |
| Lactobacilli, culture-positive only | 22 | 5.30 ± 1.34 | $1.99 \times 10^5$ | 12 | 3.34 ± 0.54 | $2.20 \times 10^3$ | 1.96 | 1.69 | <0.001 | <0.001 |
| Mutans streptococci, all children (non-detects = ½ LOD) | 30 | 3.76 ± 2.25 | $5.78 \times 10^3$ | 26 | 2.02 ± 0.93 | $1.05 \times 10^2$ | 1.74 | 0.97 | <0.001 | <0.001 |
| Lactobacilli, all children (non-detects = ½ LOD) | 30 | 4.34 ± 1.98 | $2.19 \times 10^4$ | 26 | 2.46 ± 0.91 | $2.87 \times 10^2$ | 1.88 | 1.18 | <0.001 | <0.001 |

log10 CFU/mL, mean ± SD; geometric mean in CFU/mL. 'All children' analyses set non-detects to half the detection floor ($5 \times 10^1$ CFU/mL). Hedges g, standardised mean difference. P from Welch's *t*-test and Mann–Whitney U test (MWU).

**Table 5.** Adjusted associations of CHD status with microbiological and caries outcomes (maximum likelihood).

| Model | Specification | *n* | Estimate for CHD | 95% CI | *p* |
|---|---|---|---|---|---|
| Mutans streptococci detected: crude | sm_pos ~ chd | 56 | 8.76 | 2.16–35.56 | 0.002 |
| Mutans streptococci detected: age-adjusted | sm_pos ~ chd + age | 56 | 10.53 | 2.47–44.89 | 0.001 |
| Mutans streptococci detected: age, brushing, sugar | sm_pos ~ chd + age + brush_daily + sugar_3plus | 56 | 44.73 | 3.94–508.13 | 0.002 |
| Lactobacilli detected: crude | lb_pos ~ chd | 56 | 3.21 | 1.05–9.81 | 0.041 |
| Lactobacilli detected: age-adjusted | lb_pos ~ chd + age | 56 | 3.22 | 1.03–10.04 | 0.044 |
| Lactobacilli detected: age, brushing, sugar | lb_pos ~ chd + age + brush_daily + sugar_3plus | 56 | 3.46 | 1.05–11.45 | 0.042 |
| Both organisms detected: age-adjusted | both_pos ~ chd + age | 56 | 27.34 | 3.14–238.15 | 0.003 |
| Any treated tooth: age- and dmft-adjusted | treated ~ chd + age + dmft | 56 | 11.38 | 2.35–55.08 | 0.002 |
| Lactobacilli log10 CFU/mL (all children): age, brushing, sugar | log_lb_all ~ chd + age + brush_daily + sugar_3plus | 56 | β 1.98 | 1.07–2.89 | <0.001 |

**Table 5.** *Cont.*

| Model | Specification | *n* | Estimate for CHD | 95% CI | *p* |
|---|---|---|---|---|---|
| Mutans streptococci log10 CFU/mL (all children): age, brushing, sugar | log_sm_all ~ chd + age + brush_daily + sugar_3plus | 56 | β 2.14 | 1.26–3.02 | <0.001 |
| dmft: age-adjusted | dmft ~ chd + age (NB, α = 0.35) | 56 | IRR 0.99 | 0.68–1.44 | 0.949 |
| dmft: age + organism status | dmft ~ chd + age + sm_pos + lb_pos (NB, α = 0.35) | 56 | IRR 1.03 | 0.67–1.58 | 0.909 |
| dmft: full model | dmft ~ chd + age + brush_daily + sugar_3plus + sm_pos + lb_pos (NB, α = 0.35) | 56 | IRR 1.00 | 0.63–1.59 | 0.995 |

Logistic models report odds ratios; linear models report the difference in log10 CFU/mL (β); negative-binomial (NB) models report incidence rate ratios (IRR) with the profile-likelihood dispersion α. brush_daily, tooth-brushing at least once daily; sugar_3plus, free-sugar exposure ≥ 3 times/day; sm_pos/lb_pos, culture-positive for mutans streptococci/lactobacilli.

### *3.7. Sensitivity Analyses and Power*

Restricting the analysis to the 26 cases and 26 controls within the protocol age range, to the 26 cases without chronic medication, to the 53 children with verified dmft arithmetic, or to the 52 children at their first dental visit did not change any conclusion (Table S1): the OR for mutans streptococci detection ranged from 8.3 to 9.9 and the lactobacillus load difference from 1.76 to 1.96 log10 units in every subset, while dmft remained equal between groups ($p \geq 0.83$). The flagged records are listed in Table S2 and carriage by age band in Table S5. The width of the intervals shows where this sample is informative and where it is not: the interval for mutans streptococci detection excludes no difference, while the intervals for lactobacillus detection and for dmft are wide enough to contain both no difference and a difference that would matter ($d = 0.5$ for dmft) (Table S4).

### *3.8. Robustness to Sparse Data, Censoring, and Unmeasured Confounding*

Firth penalised regression gave smaller and more stable estimates than maximum likelihood without changing any conclusion (Figure 5a, Table 6). The OR for mutans streptococci detection was 7.64 (95% profile CI 2.22–33.4) crude, 8.87 (2.49–40.3) after adjustment for age, and 22.8 (4.16–274) after further adjustment for brushing and sugar exposure, against a maximum-likelihood estimate of 44.7 for the last model; the corresponding ORs for lactobacilli were 3.07 (1.05–9.50), 3.01 (1.02–9.41) and 3.09 (1.02–10.1; $p = 0.046$), and for detection of both organisms 14.9 (3.19–146), 17.9 (3.70–179), and 27.4 (4.39–447). For lactobacilli at $\geq 10^5$ CFU/mL, where no control was positive and maximum likelihood had no finite solution, the penalised OR was 27.2 (3.16–3567; $p < 0.001$).

The left-censored model for lactobacillus load, which was identifiable because 46% of controls were culture-positive, gave a larger adjusted difference than the half-floor substitution: 2.59 log10 CFU/mL (1.35–3.83) against 1.98 (1.07–2.89), with intermediate values in the crude and age-adjusted models (Figure 5b, Table 6). The censored-normal fits placed the mean log10 lactobacillus load at 4.06 (SD 2.41) in CHD and 2.00 (SD 1.43) in controls, so that the substitution model, by pulling every non-detect to the same value, understated the separation between groups. The model was not identifiable for mutans streptococci, for which only 11.5% of controls were positive, and the substitution estimate (2.14 log10, 1.26–3.02) is reported for that organism.

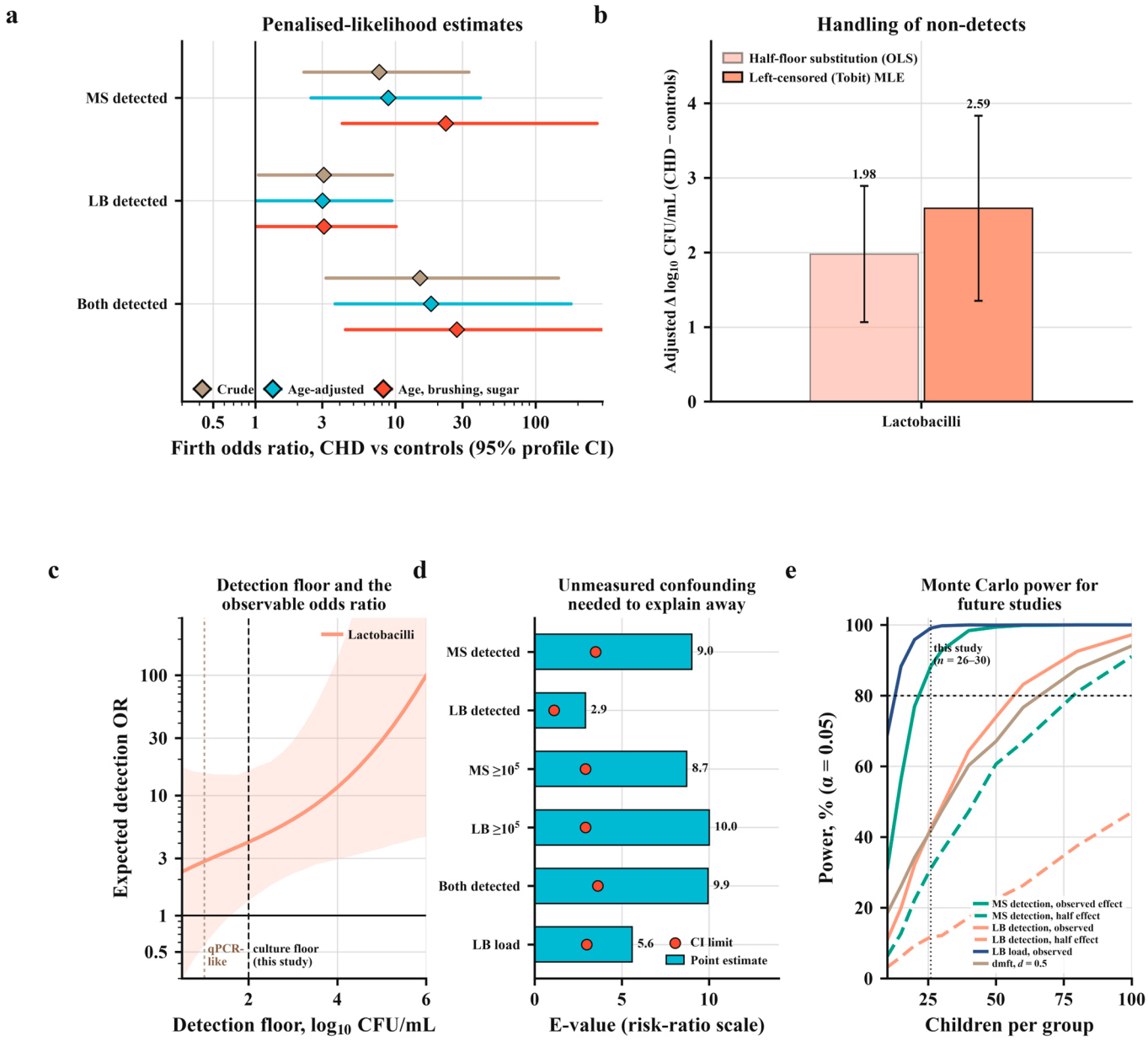



**Figure 5.** Robustness analyses and simulation. (**a**) Firth penalised-likelihood odds ratios for CHD with 95% profile-likelihood confidence intervals, crude, age-adjusted, and adjusted for age, daily brushing, and frequent sugar exposure, for detection of mutans streptococci (MS), lactobacilli (LB), and both organisms. (**b**) Adjusted difference in log10 lactobacillus load (CHD minus controls) from the half-floor substitution model and from the left-censored (Tobit) model, with 95% confidence intervals; the censored model was not identifiable for mutans streptococci. (**c**) Expected detection odds ratio for lactobacilli as a function of the assay detection floor, from censored-normal fits to each group; line, point estimate; band, 95% simulation interval from 4000 parameter draws; dashed lines mark a molecular-assay floor ($10^1$ CFU/mL) and the culture floor of this study ($10^2$ CFU/mL). (**d**) E-values for the fully adjusted associations on the risk-ratio scale: bars, strength of unmeasured confounding needed to explain away the point estimate; circles, to move the confidence limit to the null. (**e**) Monte Carlo power (2000 simulated datasets per point, $\alpha = 0.05$) against children per group for the observed effects and for effects half as large; horizontal line, 80% power; vertical line, the group size of this study. In (**a**) taupe denotes the crude model, blue the age-adjusted model and red the model adjusted for age, daily brushing and frequent sugar exposure. In (**b**) the pale bar is the half-floor substitution and the solid bar the left-censored model, both for lactobacilli. In (**e**) solid lines are the observed effects and dashed lines the effects halved.

**Table 6.** Robustness analyses: Firth penalised regression, left-censored regression, E-values, permutation tests, and bootstrap intervals.

| Analysis | Outcome | Model | Estimate (95% CI) | *p* | Comparison |
|---|---|---|---|---|---|
| Firth logistic regression | Mutans streptococci detected | crude | OR 7.64 (2.22–33.38) | <0.001 | ML: 8.76 (2.16–35.56) |
| Firth logistic regression | Mutans streptococci detected | age-adjusted | OR 8.87 (2.49–40.34) | <0.001 | ML: 10.53 (2.47–44.89) |
| Firth logistic regression | Mutans streptococci detected | fully adjusted | OR 22.81 (4.16–273.87) | <0.001 | ML: 44.73 (3.94–508.13) |
| Firth logistic regression | Lactobacilli detected | crude | OR 3.07 (1.05–9.50) | 0.040 | ML: 3.21 (1.05–9.81) |
| Firth logistic regression | Lactobacilli detected | age-adjusted | OR 3.01 (1.02–9.41) | 0.045 | ML: 3.22 (1.03–10.04) |
| Firth logistic regression | Lactobacilli detected | fully adjusted | OR 3.09 (1.02–10.09) | 0.046 | ML: 3.46 (1.05–11.45) |
| Firth logistic regression | Mutans streptococci $\geq 10^5$ CFU/mL | crude | OR 8.71 (1.81–85.50) | 0.005 | ML: 12.50 (1.47–106.04) |
| Firth logistic regression | Mutans streptococci $\geq 10^5$ CFU/mL | age-adjusted | OR 10.73 (2.15–107.99) | 0.002 | ML: 15.96 (1.80–141.39) |
| Firth logistic regression | Mutans streptococci $\geq 10^5$ CFU/mL | fully adjusted | OR 21.42 (3.10–372.88) | <0.001 | ML: 54.03 (2.90–1006.59) |
| Firth logistic regression | Lactobacilli $\geq 10^5$ CFU/mL | crude | OR 27.15 (3.16–3567.03) | <0.001 | ML: 205,558,649,546.55 (unstable) |
| Firth logistic regression | Lactobacilli $\geq 10^5$ CFU/mL | age-adjusted | OR 26.81 (3.10–3528.13) | <0.001 | ML: 204,053,118,128.02 (unstable) |
| Firth logistic regression | Lactobacilli $\geq 10^5$ CFU/mL | fully adjusted | OR 27.76 (3.09–3695.36) | <0.001 | ML: 259,056,197,849.29 (unstable) |
| Firth logistic regression | Both organisms detected | crude | OR 14.94 (3.19–145.54) | <0.001 | ML: 21.88 (2.62–182.90) |
| Firth logistic regression | Both organisms detected | age-adjusted | OR 17.92 (3.70–178.88) | <0.001 | ML: 27.34 (3.14–238.15) |
| Firth logistic regression | Both organisms detected | fully adjusted | OR 27.43 (4.39–446.80) | <0.001 | ML: 66.35 (3.99–1102.01) |
| Left-censored (Tobit) regression | Mutans streptococci log10 load | crude | not identifiable (control detection < 25%) | | Half-floor OLS: β 1.74 (0.79–2.69) |
| Left-censored (Tobit) regression | Mutans streptococci log10 load | age-adjusted | not identifiable (control detection < 25%) | | Half-floor OLS: β 1.91 (0.98–2.84) |
| Left-censored (Tobit) regression | Mutans streptococci log10 load | fully adjusted | not identifiable (control detection < 25%) | | Half-floor OLS: β 2.14 (1.26–3.02) |
| Left-censored (Tobit) regression | Lactobacilli log10 load | crude | β 2.49 (1.29–3.70) | <0.001 | Half-floor OLS: β 1.88 (1.03–2.73) |

**Table 6.** *Cont.*

| Analysis | Outcome | Model | Estimate (95% CI) | *p* | Comparison |
|---|---|---|---|---|---|
| Left-censored (Tobit) regression | Lactobacilli log10 load | age-adjusted | β 2.55 (1.32–3.79) | <0.001 | Half-floor OLS: β 1.95 (1.09–2.81) |
| Left-censored (Tobit) regression | Lactobacilli log10 load | fully adjusted | β 2.59 (1.35–3.83) | <0.001 | Half-floor OLS: β 1.98 (1.07–2.89) |
| E-value | Mutans streptococci detected | fully adjusted | approx. RR 4.78 | | E-value 9.02 (CI limit 3.49) |
| E-value | Lactobacilli detected | fully adjusted | approx. RR 1.76 | | E-value 2.91 (CI limit 1.11) |
| E-value | Mutans streptococci $\geq 10^5$ CFU/mL | fully adjusted | approx. RR 4.63 | | E-value 8.73 (CI limit 2.92) |
| E-value | Lactobacilli $\geq 10^5$ CFU/mL | fully adjusted | approx. RR 5.27 | | E-value 10.01 (CI limit 2.91) |
| E-value | Both organisms detected | fully adjusted | approx. RR 5.24 | | E-value 9.95 (CI limit 3.61) |
| E-value | Lactobacilli load (Tobit $\beta/\sigma$) | fully adjusted | approx. RR 3.07 | | E-value 5.60 (CI limit 2.99) |
| Permutation test (20,000) | MS detection, risk difference | unadjusted | observed 0.42 | 0.0015 | |
| Permutation test (20,000) | LB detection, risk difference | unadjusted | observed 0.27 | 0.0564 | |
| Permutation test (20,000) | Both detected, risk difference | unadjusted | observed 0.43 | 0.0004 | |
| Permutation test (20,000) | MS log10 load (all children), mean difference | unadjusted | observed 1.74 | 0.0008 | |
| Permutation test (20,000) | LB log10 load (all children), mean difference | unadjusted | observed 1.88 | 0.0001 | |
| Permutation test (20,000) | dmft, mean difference | unadjusted | observed −0.12 | 0.9286 | |
| Permutation test (20,000) | Treated tooth present, risk difference | unadjusted | observed 0.42 | 0.0016 | |
| Permutation test (20,000) | LB log10 load (culture-positive), mean difference | unadjusted | observed 1.96 | <0.0001 | |
| Bootstrap Hedges g (10,000) | MS log10 load, all children | unadjusted | g 0.97 (0.53–1.51) | | |
| Bootstrap Hedges g (10,000) | LB log10 load, all children | unadjusted | g 1.18 (0.71–1.82) | | |

Firth, penalised-likelihood logistic regression with profile-likelihood 95% CI and penalised likelihood-ratio P; ML, maximum likelihood. Tobit, left-censored normal regression with non-detects censored at the detection floor ($10^2$ CFU/mL); not fitted where fewer than 25% of a group was culture-positive. E-value, minimum risk-ratio association of an unmeasured confounder with both CHD and the outcome needed to explain away the fully adjusted estimate (point) or its confidence limit; odds ratios converted to approximate risk ratios by square root, standardised differences by exp(0.91 d). Permutation P from 20,000 relabellings; bootstrap percentile CI from 10,000 resamples.

Permutation tests reproduced the parametric results: the risk difference for mutans streptococci detection ($p = 0.0015$), detection of both organisms ($p = 0.0004$), the all-children load differences for mutans streptococci ($p = 0.0008$) and lactobacilli ($p = 0.0001$), the lactobacillus load difference among carriers ($p < 0.0001$), and the difference in treated-tooth prevalence ($p = 0.0016$) were all unlikely under random relabelling, whereas the lactobacillus detection difference ($p = 0.056$) and the dmft difference ($p = 0.93$) were not. Bootstrap CIs for the all-children standardised load differences excluded zero for both organisms (mutans streptococci g 0.97, 0.53–1.51; lactobacilli g 1.18, 0.71–1.82).

E-values indicated that the associations are not easily attributed to an unmeasured confounder (Figure 5d, Table 6). To explain away the fully adjusted association with mutans streptococci detection, a confounder would need to be associated with both CHD and colonisation at a risk ratio of 9.0 and of 3.5 to move the confidence limit to the null; the corresponding values were 8.7 and 2.9 for mutans streptococci at $\geq 10^5$ CFU/mL, 10.0 and 2.9 for lactobacilli at $\geq 10^5$ CFU/mL, 9.9 and 3.6 for carriage of both organisms, and 5.6 and 3.0 for lactobacillus load. The association with lactobacillus detection was the most fragile (E-value 2.9, confidence limit 1.1), consistent with its borderline significance.

### *3.9. Simulation: The Detection Floor and the Design of Future Studies*

Using the censored-normal fits for lactobacilli, the expected detection odds ratio rose monotonically with the detection floor of the assay (Figure 5c). At a floor of $10^1$ CFU/mL, typical of a molecular assay, detection would be expected in 90% of children with CHD and 76% of controls, an OR of 2.8 (95% simulation band 0.6–15.3); at the culture floor of this study, $10^2$ CFU/mL, the expected OR was 4.1 (1.3–16.1), close to the observed 3.1; at $10^3$ CFU/mL, it was 6.3 (2.3–28.2); at $10^4$ CFU/mL, it was 11.7 (3.0–138); and at the conventional high-risk threshold of $10^5$ CFU/mL, it was 28.6, with expected detection in 35% of children with CHD and 2% of controls, matching the observed 33.3% and 0%. The same two load distributions therefore yield an odds ratio near unity or one above 20 depending only on the dilution at which presence is scored.

Monte Carlo power at the group sizes of this study (26–30 per group) was 88% for the mutans streptococci detection difference with Fisher's exact test, 99% for the lactobacillus load difference, 43% for the lactobacillus detection difference, and 42% for a dmft difference of $d = 0.5$ (Figure 5e, Table S6). If the true effects were half those observed, on the log-odds or mean-difference scale, 80% power would require about 80 children per group for mutans streptococci detection, about 60 for lactobacillus load, and more than 100 for lactobacillus detection; a dmft difference of $d = 0.5$ would require about 65 per group.

## 4. Discussion

Children with CHD in this cohort were several times more likely than healthy children to carry culturable mutans streptococci and, when they carried lactobacilli, did so at roughly a hundred-fold higher density; yet, their caries experience was no greater, and a larger share of their lesions had been treated. The difference withstood penalised estimation for sparse cells, likelihood-based treatment of non-detects, permutation and bootstrap inference, exclusion of out-of-protocol records, and quantification of the confounding needed to remove it. The study cannot claim the excess is universal in CHD: the published comparisons disagree, and the simulation offers a reason why.

The dissociation between colonisation and caries experience is the opposite of what a caries-risk reading of bacterial counts predicts. Neither load correlated with dmft, and organism status did not enter the caries model. Three features of the cohort explain this. Caries experience was high in both groups, with a mean dmft near seven and fewer than one child in six caries-free, a level in keeping with hospital-based estimates of early childhood

caries in the same country [42], which leaves little room for a bacterial gradient to express itself in the index. Controls were recruited at their first visit to a dental clinic, a setting that selects children with existing dental need [39], which inflates control dmft and would mask any true excess in CHD. And children with CHD had more missing and filled teeth and a smaller untreated share, which is compatible with more of their disease having been treated, plausibly because cardiology follow-up brings families into contact with hospital dental services and because clinicians treat caries more aggressively when endocarditis is a concern. Treatment history was not recorded, and a missing tooth can also follow extraction for reasons unconnected with caries. A dmft comparison in this setting therefore measures access to treatment as much as disease, and the component analysis is more informative than the total.

The microbiological difference is unlikely to be an artefact of behaviour or of measured confounding. Children with CHD brushed at least as often as controls, received more parental help, and reported less frequent sugar exposure, the family-level factors that most often account for caries in children with special health-care needs [43,44], and adjustment for these variables did not reduce the association. An unmeasured factor would have to roughly triple the risk of both CHD and colonisation to bring the confidence limits for mutans streptococci carriage to the null, and roughly nine-fold to remove the point estimates. Antibiotic exposure, which was not recorded here and which the Swedish infant cohort found to be far more frequent in CHD [24], is the most plausible candidate of that magnitude, and it would act by reshaping the oral community, a mechanism of the exposure itself. The other candidate explanations are the ones that have been proposed for CHD generally: lower salivary flow and buffering capacity, which the Indian cross-sectional study measured directly and found reduced in CHD alongside a 1.6 log10 higher mutans streptococci count [45]; and chronic medication, which affected only four children here and whose exclusion did not alter the result. Lactobacilli are aciduric organisms whose density tracks the availability of low-pH retentive niches, and a hundred-fold difference in their load with equal numbers of decayed teeth is consistent with niches that are more acidic or more retentive in children with CHD. Neither salivary composition nor the pH of these niches was measured here, so this account stands as a hypothesis and not as a finding of the study. Saliva composition would be the most economical explanation [46] and should be measured with the culture-independent salivary profile that separates caries-active from caries-free children [47,48] in any extension of this work.

The simulation clarifies why the literature has appeared contradictory. Detection is method-dependent: qPCR identified *S. mutans* in 87.5% and 88.7% of Sudanese children [29] and in 95% and 100% of Turkish children [30], leaving no room for a between-group difference, whereas selective culture at a detection limit near $10^3$ CFU/mL identified mutans streptococci in 12% to 84% of controls across studies [23–28]. A molecular assay answers whether the organism is present; a culture at a fixed dilution answers whether it is present above a density threshold, and only the second can differ between groups when the organism is near-universal. The censored-normal model fitted to this cohort shows that the same pair of load distributions gives an expected odds ratio of about 3 at a molecular floor, 4 at the culture floor used here, and nearly 30 at the $10^5$ CFU/mL threshold used for caries-risk classification. Load comparisons in the literature are further confounded by scale: three studies reported arithmetic CFU means with standard deviations larger than the means [26,30,49], dominated by a few heavily colonised children; in one, the control standard deviation was implausibly small [23]. Control selection varied from siblings [26,50] and school children [29] to attendees of dental clinics [28,45], a difference that affects the caries comparison directly and the microbiological comparison through it. Differences in floor, scale, and comparison group give the most economical reading of the

conflicting reports, and the 2024 pooled dmft estimate, with no significant difference and high heterogeneity [33], fits the same pattern.

The methodological results have implications beyond this cohort. Half-floor substitution understated the lactobacillus difference by about 0.6 log10 units relative to the censored model, and maximum likelihood overstated the fully adjusted mutans streptococci odds ratio by a factor of two relative to Firth's penalisation; neither reversed a conclusion here, but both are of the size that decides significance at this scale, and both are avoidable. Sample-size calculation for a future study, a different exercise from power computed after this one, indicates that the 25 to 40 children per group usual in this field would detect a difference in detection of the size seen here but not one half as large, and not the caries comparison most studies treat as primary. Settling the question would need substantially larger groups, confirmed identification, and loads reported against a stated detection floor.

For clinical practice, the implication is specific. Current endocarditis-prevention guidance rests on oral health rather than prophylaxis [9,11], oral health in a child with CHD is usually judged by caries status at a dental visit, and the clinical guidelines on oral care for children with special health-care needs vary in quality [51]. This cohort shows that caries status can be equal while the density of acidogenic and aciduric organisms is one to two orders of magnitude higher. In this cohort, a treated dentition did not mark a child with a low bacterial burden. Whether microbiological measurement should be added to clinical examination cannot be settled here, because neither bacteraemia nor infective endocarditis was assessed and no child was followed for a clinical event. Whether that density is the quantity endocarditis prevention should target is a question for studies with clinical endpoints. The results do not bear on antibiotic prophylaxis, which should continue to follow cardiac risk category and procedure type.

This study has limitations that constrain these conclusions. Identification was by colony morphology on selective media without confirmation, so the organisms are presumptive mutans streptococci and lactobacilli and no statement about species can be made. The counts describe organisms with the colony phenotype on each selective medium, and any non-target organism able to grow there was counted with them; such misclassification, if equal in both groups, would narrow the observed difference and could not create it. The sample was small, controls were drawn from a dental clinic, four cases fell outside the protocol age range, and three records contained transcription errors, although none of the sensitivity analyses that addressed these issues altered the findings. Cardiac lesion type, surgical status, cyanosis, and antibiotic exposure were not recorded, and salivary flow, pH, and buffering were not measured. The censored-normal model could not be fitted for mutans streptococci because too few controls were positive, and the detection-floor simulation therefore rests on lactobacilli alone and on the assumption that log10 load is normally distributed within each group, including among children whose counts fell below the floor. The E-value quantifies the confounding that would be required but cannot say whether it exists. The study had adequate power for the differences in mutans streptococci detection and lactobacillus load but not for the difference in lactobacillus detection or for a moderate difference in dmft, which should be read as inconclusive, not as null.

## 5. Conclusions

Infective endocarditis prevention in children with CHD is built on the premise that oral bacterial burden can be controlled through oral health, and that premise is usually assessed by looking for caries. In the cohort studied here, children with CHD carried culturable mutans streptococci eight to ten times more often than healthy children and, among carriers, a hundred-fold higher lactobacillus load, while their caries experience was identical and more of their lesions had been treated. The excess colonisation survived adjustment for

age, brushing, and sugar exposure, penalised and censored re-estimation, permutation inference, and every sensitivity analysis, and would require an unmeasured confounder of a strength rarely seen in oral epidemiology to be explained away. Simulation from the fitted load distributions shows that the odds ratio a study observes depends steeply on the detection floor of its assay, which accounts for much of the disagreement among published comparisons. These findings are preliminary. They come from a single small clinic-based sample with presumptive identification, and they describe colonisation, not bacteraemia or infective endocarditis, neither of which was assessed here. What they support is a specific next step: that cariogenic burden in children with CHD be measured directly in larger studies, with confirmed identification, logarithmic quantification against a stated detection floor, salivary physiology, population-based controls, and 80 to 100 children per group, before the microbiological basis of endocarditis prevention in this group can be regarded as established.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms14122770/s1, 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 × 104 CFU/mL. Photographs were taken during the study and are reproduced from the laboratory records without processing other than cropping. Table S1: Sensitivity analyses of the primary outcomes. Table S2: Records flagged at data verification. Table S3: Relationship between colonisation and caries experience. Table S4: Analytic power and sample-size considerations. Table S5: Detection and caries experience by age band. Table S6: Monte Carlo power (proportion of 2000 simulated studies with $p < 0.05$) by children per group, for the observed effects and for effects half as large. File S1: Blank parental informed consent form, the English version of the form approved by the Research Ethics Committee of the Qassim Health Cluster, carrying no participant name, signature or date. The cleaned participant-level dataset and all analysis code, including the robustness and simulation scripts, are provided in the same archive.

**Author Contributions:** Conceptualization, S.S.A. and K.S.A.; methodology, S.S.A., S.A.-H.A., and K.S.A.; software, M.A.; validation, A.A. and M.U.A.; formal analysis, M.A. and S.A.-H.A.; investigation, T.S.A., S.S.A., and F.A.; resources, K.S.A. and A.A.; data curation, T.S.A. and M.U.A.; writing—original draft preparation, S.S.A. and T.S.A.; writing—review and editing, M.A., S.A.-H.A., F.A., A.A., M.U.A., and K.S.A.; visualization, M.A.; supervision, S.S.A. and K.S.A.; project administration, S.S.A.; funding acquisition, K.S.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the Qassim Health Cluster, Saudi Arabia (protocol code H-04-Q-001, date of approval 4 October 2023).

**Informed Consent Statement:** Written informed consent was obtained from a parent or legal guardian of every child who participated in the study, on the parental consent form approved by the Research Ethics Committee of the Qassim Health Cluster. The form was explained and signed in Arabic, and a blank copy of it, carrying no participant name or signature, is provided as File S1 in the Supplementary Materials.

**Data Availability Statement:** The cleaned participant-level dataset and all analysis code, including the robustness and simulation scripts, are available in the Supplementary Materials. GBD 2023 estimates are available from the Institute for Health Metrics and Evaluation (https://vizhub.healthdata.org/gbd-results/ (accessed on 5 November 2026)).

**Acknowledgments:** The authors would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University (https://www.qu.edu.sa) for financial support (QU-APC-2026). The

authors thank the children and their parents who took part in this study and the staff of the paediatric cardiology and paediatric dentistry clinics at Qassim University for their help with recruitment and sample collection.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| CFU | colony-forming units |
| CHD | congenital heart disease |
| CI | confidence interval |
| dmft | decayed, missing and filled primary teeth |
| GBD | Global Burden of Disease |
| IQR | interquartile range |
| IRR | incidence rate ratio |
| LB | lactobacilli |
| LOD | limit of detection |
| MRS | de Man–Rogosa–Sharpe agar |
| MS | mutans streptococci |
| MSB | Mitis Salivarius Bacitracin agar |
| NB | negative binomial |
| OR | odds ratio |
| qPCR | quantitative polymerase chain reaction |
| SD | standard deviation |
| UI | uncertainty interval |

## References

1. Institute for Health Metrics and Evaluation. *Global Burden of Disease Study 2023 (GBD 2023) Results*; Institute for Health Metrics and Evaluation: Seattle, WA, USA, 2025.
2. Schulz-Weidner, N.; Logeswaran, T.; Jux, C.; Schlenz, M.A.; Krämer, N.; Bulski, J.C. Evaluation of the Effectiveness of an Interdisciplinary Preventive Oral Hygiene Program for Children with Congenital Heart Disease. *Int. J. Environ. Res. Public Health* **2021**, *18*, 3497. [CrossRef]
3. Mohammad, H.; Shoaib, L.A.; Dziaruddin, N.; Zahari, N. Oral health needs and barriers to care in children with congenital heart disease. *J. Clin. Pediatr. Dent.* **2025**, *49*, 82. [CrossRef]
4. Rao, P.S. Management of Congenital Heart Disease: State of the Art—Part II—Cyanotic Heart Defects. *Children* **2019**, *6*, 54. [CrossRef]
5. Saraç, F.; Derelioğlu, S.Ş.; Şengül, F.; Laloğlu, F.; Ceviz, N. The Evaluation of Oral Health Condition and Oral and Dental Care in Children with Congenital Heart Disease. *J. Clin. Med.* **2023**, *12*, 3674. [CrossRef]
6. Seow, W.K. Early Childhood Caries. *Pediatr. Clin. N. Am.* **2018**, *65*, 941–954. [CrossRef]
7. Pitts, N.B.; Zero, D.T.; Marsh, P.D.; Ekstrand, K.; Weintraub, J.A.; Ramos-Gomez, F.; Tagami, J.; Twetman, S.; Tsakos, G.; Ismail, A. Dental caries. *Nat. Rev. Dis. Primers* **2017**, *3*, 17030. [CrossRef]
8. Foláyan, M.O.; Schroth, R.J.; Olatosi, O.; El Tantawi, M. Environmental Determinants of Early Childhood Caries: A Narrative Synthesis of Observational Evidence and Implications for Global Policy. *Dent. J.* **2025**, *13*, 484. [CrossRef]
9. Wilson, W.R.; Gewitz, M.; Lockhart, P.B.; Bolger, A.F.; DeSimone, D.C.; Kazi, D.S.; Couper, D.J.; Beaton, A.; Kilmartin, C.; Miro, J.M.; et al. Prevention of Viridans Group Streptococcal Infective Endocarditis: A Scientific Statement From the American Heart Association. *Circulation* **2021**, *143*, e963–e978. Erratum in *Circulation* **2021**, *144*, e192. [CrossRef]
10. Lockhart, P.B.; Brennan, M.T.; Sasser, H.C.; Fox, P.C.; Paster, B.J.; Bahrani-Mougeot, F.K. Bacteremia Associated With Toothbrushing and Dental Extraction. *Circulation* **2008**, *117*, 3118–3125. [CrossRef]
11. Del Giudice, C.; Vaia, E.; Liccardo, D.; Marzano, F.; Valletta, A.; Spagnuolo, G.; Ferrara, N.; Rengo, C.; Cannavo, A.; Rengo, G. Infective Endocarditis: A Focus on Oral Microbiota. *Microorganisms* **2021**, *9*, 1218. [CrossRef]
12. Selwitz, R.H.; Ismail, A.I.; Pitts, N.B. Dental caries. *Lancet* **2007**, *369*, 51–59. [CrossRef]
13. Maier, T. Oral Microbiome in Health and Disease: Maintaining a Healthy, Balanced Ecosystem and Reversing Dysbiosis. *Microorganisms* **2023**, *11*, 1453. [CrossRef]
14. Lemos, J.A.; Palmer, S.R.; Zeng, L.; Wen, Z.T.; Kajfasz, J.K.; Freires, I.A.; Abranches, J.; Brady, L.J. The Biology of *Streptococcus mutans*. *Microbiol. Spectr.* **2019**, *7*, 0051-2018. [CrossRef]

15. Caufield, P.W.; Schön, C.N.; Saraithong, P.; Li, Y.; Argimón, S. Oral Lactobacilli and Dental Caries. *J. Dent. Res.* **2015**, *94*, 110S–118S. [CrossRef]
16. Loesche, W.J. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev.* **1986**, *50*, 353–380. [CrossRef]
17. Leme, L.A.F.P.; Rizzardi, K.F.; Santos, I.B.; Parisotto, T.M. Exploring the Relationship between Salivary Levels of TNF-α, *Lactobacillus acidophilus*, *Lactobacillus gasseri*, Obesity, and Caries in Early Childhood. *Pathogens* **2022**, *11*, 579. [CrossRef]
18. Köhler, B.; Bratthall, D. Practical method to facilitate estimation of *Streptococcus mutans* levels in saliva. *J. Clin. Microbiol.* **1979**, *9*, 584–588. [CrossRef]
19. Hofmann, M.; Schulz-Weidner, N.; Krämer, N.; Hain, T. The Bacterial Oral Microbiome in Children with Congenital Heart Disease: An Extensive Review. *Pathogens* **2023**, *12*, 1269. [CrossRef]
20. Rai, K.; S, S.; Hegde, A. Oral Health Status of Children with Congenital Heart Disease and the Awareness, Attitude and Knowledge of their Parents. *J. Clin. Pediatr. Dent.* **2009**, *33*, 315–318. [CrossRef]
21. Radwan, A.N.; Felemban, O.M.; Kari, J.A.; El Desoky, S.M.; Baghlaf, K.; Elkhodhary, H.M. Dental Caries and Erosion in Children with Nephrotic Syndrome on Long-Term Liquid Medications—A Cross-Sectional Observational Study. *J. Clin. Med.* **2025**, *14*, 8669. [CrossRef]
22. Cheng, H.; Chen, R.; Milosevic, M.; Rossiter, C.; Arora, A.; Denney-Wilson, E. Interventions Targeting Bottle and Formula Feeding in the Prevention and Treatment of Early Childhood Caries, Overweight and Obesity: An Integrative Review. *Int. J. Environ. Res. Public Health* **2021**, *18*, 12304. [CrossRef]
23. Najafi, T.; Pourmoghaddas, Z.; Meskin, M.; Sabri, M.; Norousali Tehrani, M. Dental caries and gingival evaluation in children with congenital heart disease. *Int. J. Prev. Med.* **2018**, *9*, 52. [CrossRef]
24. Hansson, L.; Rydberg, A.; Stecksén-Blicks, C. Oral microfora and dietary intake in infants with congenital heart disease: A case control study. *Eur. Arch. Paediatr. Dent.* **2012**, *13*, 238–243. [CrossRef]
25. Suvarna, R.; Rai, K.; Hegde, A. Oral Health of Children with Congenital Heart Disease following Preventive Treatment. *J. Clin. Pediatr. Dent.* **2011**, *36*, 93–98. [CrossRef]
26. Franco, E.; Saunders, C.P.; Roberts, G.J.; Suwanprasit, A. Dental disease, caries related microflora and salivary IgA of children with severe congenital cardiac disease: An epidemiological and oral microbial survey. *Pediatr. Dent.* **1996**, *18*, 228–235.
27. Torres, M.C.; Ramos, M.; Coelho, T.; Harari, S. Salivary *Streptococcus mutans* and *Lactobacillus* sp. levels in cardiac children. *J. Clin. Pediatr. Dent.* **2002**, *26*, 103–109. [CrossRef]
28. Topcuoglu, N.; Bozdogan, E.; Ozsoy, S.D.; Haberal, I.; Cetin, G.; Aktoren, O.; Kulekci, G. Prevalence of Salivary *Streptococcus mutans* Serotype k in Children Undergoing Congenital Heart Surgery. *J. Clin. Pediatr. Dent.* **2013**, *38*, 175–178. [CrossRef]
29. Mohamed Ali, H.; Berggreen, E.; Nguyen, D.; Wahab Ali, R.; Van Dyke, T.E.; Hasturk, H.; Mustafa, M. Dental plaque microbial profiles of children from Khartoum, Sudan, with congenital heart defects. *J. Oral Microbiol.* **2017**, *9*, 1281556. [CrossRef]
30. Aksakal, S.D.; Guven, Y.; Topcuoglu, N.; Kulekci, G.; Aktoren, O. Assessment of oral bacteria potentially associated with the mobile microbiome in children with congenital heart disease. *J. Clin. Pediatr. Dent.* **2024**, *48*, 47. [CrossRef]
31. Schulz-Weidner, N.; Weigel, M.; Turujlija, F.; Komma, K.; Mengel, J.P.; Schlenz, M.A.; Bulski, J.C.; Krämer, N.; Hain, T. Microbiome Analysis of Carious Lesions in Pre-School Children with Early Childhood Caries and Congenital Heart Disease. *Microorganisms* **2021**, *9*, 1904. [CrossRef]
32. Karikoski, E.; Sarkola, T.; Blomqvist, M. Dental caries prevalence in children with congenital heart disease—A systematic review. *Acta Odontol. Scand.* **2021**, *79*, 232–240. [CrossRef]
33. Moussa, C.; Savard, G.; Estrade, L.; Bourgi, R.; Kharouf, N.; Denis, F.; Daou, M.H. Dental Health in Children with Congenital Heart Defects: A Systematic Review and Meta-Analysis. *J. Clin. Med.* **2024**, *13*, 7022. [CrossRef]
34. Helsel, D.R. Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it. *Chemosphere* **2006**, *65*, 2434–2439. [CrossRef]
35. Firth, D. Bias reduction of maximum likelihood estimates. *Biometrika* **1993**, *80*, 27–38. [CrossRef]
36. Heinze, G.; Schemper, M. A solution to the problem of separation in logistic regression. *Stat. Med.* **2002**, *21*, 2409–2419. [CrossRef]
37. World Health Organization. *Oral Health Surveys: Basic Methods*, 5th ed.; World Health Organization: Geneva, Switzerland, 2013.
38. Cheever, V.J.; Mohajeri, A.; Patel, K.; Burris, R.C.; Hung, M. Impact of Free Sugar Consumption on Dental Caries: A Cross-Sectional Analysis of Children in the United States. *Dent. J.* **2025**, *13*, 48. [CrossRef]
39. Mallineni, S.K.; Alassaf, A.; Almulhim, B.; Alghamdi, S. Influence of Tooth Brushing and Previous Dental Visits on Dental Caries Status among Saudi Arabian Children. *Children* **2023**, *10*, 471. [CrossRef]
40. Tobin, J. Estimation of Relationships for Limited Dependent Variables. *Econometrica* **1958**, *26*, 24. [CrossRef]
41. VanderWeele, T.J.; Ding, P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann. Intern. Med.* **2017**, *167*, 268–274. [CrossRef]
42. Thirunavukkarasu, A.; Alaqidi, S.F. Early Childhood Caries—Prevalence, Associated Factors, and Severity: A Hospital-Based Study in Riyadh, Saudi Arabia. *Healthcare* **2024**, *12*, 1376. [CrossRef]

43. Anwar, D.S.; Mohd Yusof, M.Y.P.; Ahmad, M.S.; Md Sabri, B.A. Family Influences on the Dental Caries Status of Children with Special Health Care Needs: A Systematic Review. *Children* **2022**, *9*, 1855. [CrossRef]
44. Zaborskis, A.; Kavaliauskienė, A.; Razmienė, J.; Razmaitė, A.; Andruškevičienė, V.; Narbutaitė, J.; Bendoraitienė, E.A. Pathways Between Parental Attitudes and Early Childhood Caries in Preschool Children. *Dent. J.* **2025**, *13*, 205. [CrossRef]
45. Dhinsa, K.; Godhi, B.S.; Saha, S.; Singh, A.; Sarkar, A.; Singh, A. Assessment of oral health, salivary alterations, and parental KAP in children with congenital heart disease. *J. Oral Biol. Craniofacial Res.* **2026**, *16*, 101504. [CrossRef]
46. Ștefârță, A.; Brătoiu, M.R.; Rădoi, M.A.; Mercuț, V.; Ionescu, M.; Scrieciu, M.; Petcu, I.-C.; Mărășescu, P.-C.; Amărăscu, M.O.; Popescu, A.M.; et al. Assessment of Salivary Parameters—pH, Buffering Capacity and Flow-Associated with Caries Susceptibility. *Diagnostics* **2026**, *16*, 625. [CrossRef]
47. Jiang, S.; Gao, X.; Jin, L.; Lo, E. Salivary Microbiome Diversity in Caries-Free and Caries-Affected Children. *Int. J. Mol. Sci.* **2016**, *17*, 1978. [CrossRef]
48. Lee, E.; Park, S.; Um, S.; Kim, S.; Lee, J.; Jang, J.; Jeong, H.-o.; Shin, J.; Kang, J.; Lee, S.; et al. Microbiome of Saliva and Plaque in Children According to Age and Dental Caries Experience. *Diagnostics* **2021**, *11*, 1324. [CrossRef]
49. Rosén, L.; Rydberg, A.; Sjöström, I.; Stecksén-Blicks, C. Saliva profiles in children using heart failure medication: A pilot study. *Eur. Arch. Paediatr. Dent.* **2010**, *11*, 187–191. [CrossRef]
50. Hallett, K.B.; Radford, D.J.; Seow, W.K. Oral health of children with congenital cardiac diseases: A controlled study. *Pediatr. Dent.* **1992**, *14*, 224–230.
51. Alamri, H.; Alshammari, F.R.; Rahmah, A.B.; Aljohani, M. Quality of Clinical Guidelines on Oral Care for Children with Special Healthcare Needs: A Systematic Review. *Int. J. Environ. Res. Public Health* **2023**, *20*, 1686. [CrossRef]


**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.