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Are Mutans Streptococci Detected in Preschool Children a Reliable Predictive Factor for Dental Caries Risk? A Systematic Review

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Key Words
Caries risk, indicators · Mutans streptococci · Preschool children

Abstract
Research suggests that mutans streptococci play an important role in cariogenesis in children but the usefulness of bacterial testing in risk assessment is unknown. Our objective was to summarize the literature assessing the association of mutans streptococci and dental caries in preschool children, (Pre)Medline (1966–2003), Embase (1980–2003), the Cochrane Register of Controlled Trials (2003, issue 3), and reference lists of included studies were searched. All abstracts found by the electronic searches (n = 981) were independently scrutinized by 2 reviewers. Minimal requirements for inclusion were assessment of preschool children without caries at baseline, reporting of mutans streptococci present in saliva or plaque at baseline and assessment of caries presence after a minimum of 6 months of follow-up. Participants' details, test methods, methodological characteristics and findings were extracted by one reviewer and cross-checked by another. Homogeneity was tested using χ^2 tests. Results of plaque and saliva testing were pooled separately using a fixed effects model. Methodological quality of reports was low. Out of 9 studies included, data from 3 reports on plaque test assessment alone (n = 300) and from 4 reports on saliva test assessment alone (n = 451) were available for pooled analysis. The pooled risk ratio (95% CI) was 3.85 (2.48–5.96) in studies using plaque tests and 2.11 (1.47–3.02) in those using saliva testing. Presence of mutans streptococci, both in plaque or saliva of young caries-free children, appears to be associated with a considerable increase in caries risk. Lack of adjustment for potential confounders in the original studies, however, limits the extent to which interpretations for practice can be made.

Dental caries in preschool children is again a relevant health care problem in Western countries [Haugejorden and Birkeland, 2002; Pitts et al., 2003]. While timely provision of prophylactic prevention programmes including salt fluoridation, the use of fluoridated dentifrices and dental education led to a substantial reduction in caries development (in Switzerland) between the 1970s and

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1990s [Menghini et al., 2003a; Steiner et al., 1995], recent surveys report a remarkable re-increase of caries especially in deciduous teeth and particularly in young children over the last decade [Menghini et al., 2003a, b]. Reasons for this increase are not fully understood but may partly be explained by migration. In Switzerland for example the mean dmft values of Swiss schoolchildren were 4 times lower than those from schoolchildren of former Yugoslavia and Albania [Menghini et al., 2003b].

Tooth decay in the primary dentition is particularly worrying, first because it may lead to problems of dentition development and second, because children with rampant caries regularly require treatment under general anaesthesia. Furthermore, early caries may trigger caries in the permanent dentition. Commonly accepted risk indicators for dental caries in children comprise low socioeconomic status, poor oral hygiene in parents and children, lack of fluoride exposure, dietary habits (e.g. sweet snacks) and an abnormal saliva secretion rate [Messer, 2000; Reich et al., 1999; Tinanoff et al., 2002]. Furthermore, the importance of microbiological factors has been recognised. Infection with mutans streptococci seems to play an important role in cariogenesis mainly because these bacteria are acidogenic, aciduric and produce specific intra- and extracellular polysaccharides that facilitate microbial adherence on teeth.

Simple test kits are available that allow identification of infected individuals, but their usefulness in risk assessment for children is largely unknown. Numerous studies have quantified the association between the detection of bacteria and caries development, but these studies are generally small, scattered and not easy to access [Alaluusua and Renkonen, 1983; O'Sullivan and Thibodeau, 1996; Twetman et al., 1994]. We therefore performed a systematic review using robust methodology and studying all available research assessing the association of mutans streptococci detected in 2- to 5-year-old preschool children and the subsequent development of caries. We chose a minimum age of 2 years because several articles reported that children commonly acquire mutans streptococci between the 1st and 3rd years of life [Caufield et al., 1993; Wendt et al., 1996].

Materials and Methods

Data Sources

We searched the electronic databases Medline and Medline In-Process, Embase and the Cochrane Central Register of Controlled Trials (all Ovid® Version) from inception to December 2003 and the Dental GPRS database (Adivan High Tech AG, Wangen, Switzerland, 2003) without language restrictions for relevant articles using combinations of the Medical Subject Heading (MeSH) terms 'tooth diseases' and 'streptococcus', and the (truncated) text words 'caries' and 'streptococcus'. Searches were limited to infants, child, preschool child, schoolchild or adolescent. The Dental GPRS database was searched using the key words 'mutans' and 'caries'. The search strategy for Medline is as shown: 1 exp dental caries; 2 exp streptococcus mutans; (1 and 2); (caries and streptococcus).af.; (3 or 4); limit 5 to all child <0 to 18 years>.

Reviewing the reference lists of included articles complemented the searches. Manufacturers of salivary mutans streptococci tests (Ivoclar Vivadent AG, Schaan, Liechtenstein; Orion Diagnostica, Espoo, Finland; Hain Diagnostika GmbH, Nehren, Germany) were contacted for any additional unpublished data. Authors of reports that fulfilled the inclusion criteria but did not provide enough information for this review were contacted for additional data.

Study Selection

All observational studies performed in populations consisting of children between 2 and 5 years of age at baseline and younger than 16 years at the final examination were included. Children had to have no caries or previous caries experience and had to be tested for mutans streptococci in plaque or saliva at the beginning of the study. Minimal follow-up required was 6 months after baseline investigation. Furthermore, the studies had to report the proportion of individuals that developed caries and if possible the number of carious lesions (including white spots), missing teeth and filled teeth of the primary as well as of the permanent dentition as a measure of the development of caries. The lesions were detected by visual methods, probing, X-rays, electronic conductance measuring or laser fluorescence. Only cohort studies were selected.

Two reviewers (N.T., J.S.) independently assessed all identified titles and abstracts for inclusion. Any discrepancies were resolved by discussion. All articles considered as possibly relevant were obtained in full-text and read by one reviewer (N.T.). Uncertainties about inclusion were discussed with a second reviewer (J.S.), who had read the respective article.

Methodological Assessment

Each study was assessed methodologically using a validation checklist for observational studies [Altman, 2001; Downs and Black, 1998] (Appendix). In order to collect a complete list of potential confounders that should be assessed in such a study we performed an extensive literature search and interviewed three experts in caries research in Germany and Switzerland. They suggested the following confounders: oral hygiene; dietary factors; tooth morphology; microbiological factors; fluoride exposure; saliva; general health conditions; socio-economic status; parent's oral hygiene and dental health; living area; life events; age; sex; race. Based on these predefined criteria one reviewer (N.T.) carefully extracted the data and these were then cross-checked by a second reviewer (L.M.B.).

Statistical Analysis

Outcome data from each of the included studies were abstracted into 2 × 2 tables. Heterogeneity (i.e. differences between studies) of risk ratios was assessed graphically using forest plots and statistically using χ^2 tests to decide on how to proceed with quantitative synthesis. This formal statistical analysis examined whether the observed variation in study results was compatible with the variation expected by chance alone. Exploration of the causes of

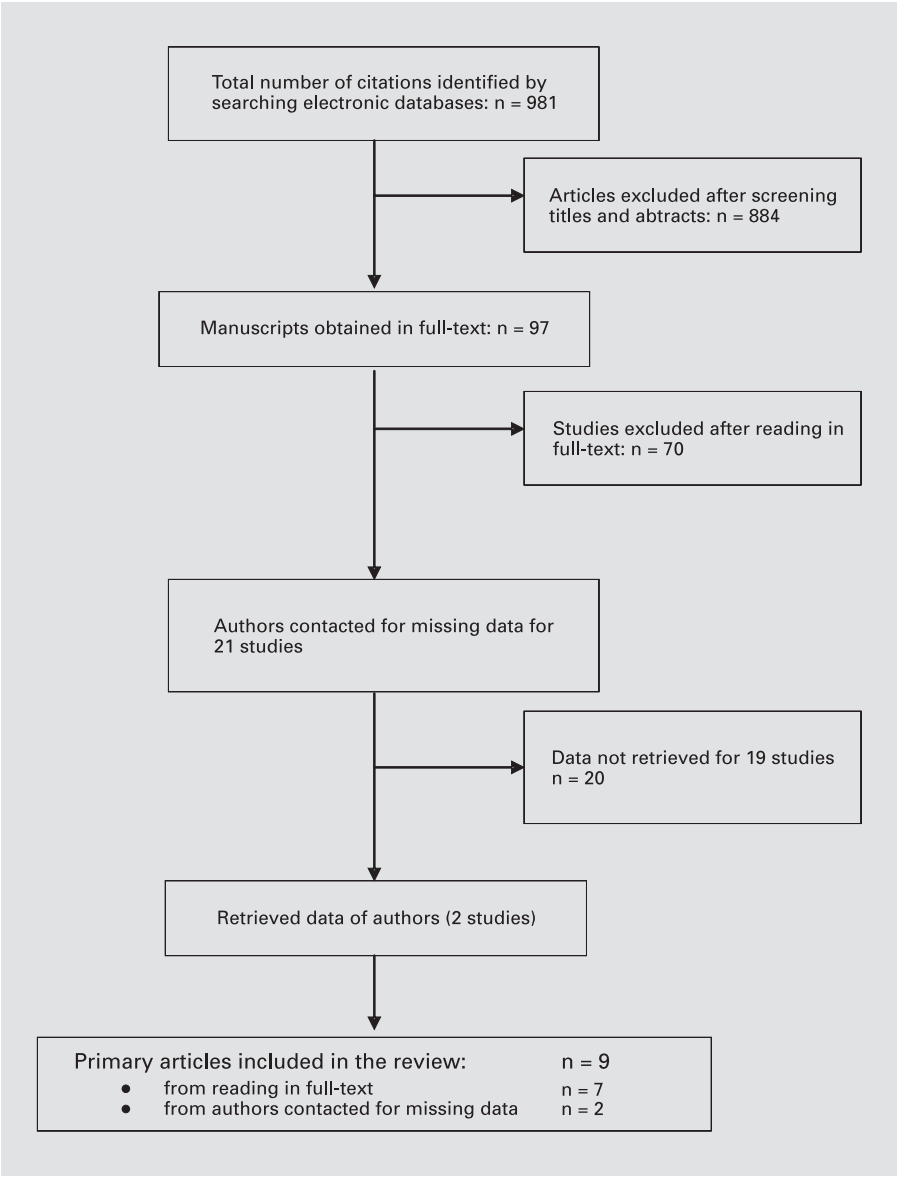

Fig. 1. Study selection process for systematic review on reliability of mutans streptococci as prognostic indicator for caries risk prediction. For primary articles included in the review, see table 1.

heterogeneity was planned using variation in features of the population (inclusion and exclusion criteria), threshold for positive test (test definition) and study quality (methodological heterogeneity). If appropriate, we planned to perform a meta-analysis where risk ratios from individual studies would be pooled stratified for the mutans streptococci detection method applied (testing), if no heterogeneity was detected. No pooling would be undertaken in the presence of significant source heterogeneity. The extracted data were entered into an Excel Work sheet (Office 2000® Microsoft Corp., Redmond, Wash., USA). After a consistency check (L.M.B.), data were transferred and analysed with the Stata statistical software package (Release 8.2, Stata® Statistical Software, StataCorp. 2004, College Station, Tex., USA).

Results of Literature Search

Electronic searches retrieved 981 non-duplicate records. 97 articles were read in full-text and out of these, 7 studies [Alaluusua and Renkonen, 1983; O'Sullivan and Thibodeau, 1996; Pienihäkkinen and Jokela, 2002; Rodriguez Miro et al., 1989; Seki et al., 2003b; Tenovuo et al., 1990; Twetman et al., 1994] fulfilled the inclusion criteria. The detailed study flow is described in figure 1. In 2 studies [Rodriguez Miro et al., 1989; Twetman et al., 1994] only data of the mutans-negative subgroup of children were used for analyses. 23 first or corresponding

Table 1. Characteristics of included studies

Author year	Country	Study design	Plaque or saliva test	Sample size	Age years	Confounding factors considered	Population	Follow-up years
Alaluusua and Renkonen [1983]	Finland	cohort study	plaque and saliva	39	2	not reported	mixed socio-economic populations of two suburbs	2
Litt et al. [1995]	USA	cohort study	saliva	183	3–4	low income; sugar intake; baby bottle use; tooth brushing habits; parents' education; life events; race; age	parents with low income	1
O'Sullivan and Thibodeau [1996]	USA	cohort study	saliva	88	3.8	optimally fluoridated tap water; low socio-economic status	Head Start children	2
Pienihäkkinen and Jokela [2002]	Finland	cohort study	plaque	206	2	no fluoridated tap water	all children born in 1987/88	3
Rodriguez Miro et al. [1989]	Cuba	cohort study	saliva	117	2–3	not reported	not reported	1
Seki et al. [2003a]	Japan	cohort study	plaque and saliva	129	3.8 (mean)	no fluoridated area; visible plaque	attending two nursery schools in Tokyo, Japan	1
Tenovuo et al. [1990]	Finland	cohort study	plaque	24	1.9 (mean)	no fluoridated tap water	13 from Turku University Central Hospital because of recurrent otitis media or other repeated upper respiratory tract infections, others randomly selected from children attending regular check-ups	2.7 (1.0–3.6)
Twetman et al. [1994]	Sweden	cohort study	saliva	136	4.5	low content of fluoride in tap water (0.1ppm); all used fluoridated toothpaste	attending kindergartens in Halmstad, Sweden	2
Wendt et al. [1996]	Sweden	cohort study	saliva	110	2	oral hygiene; dietary factors; visible plaque; immigrant status; parents' education; life events	all children living in defined area	1

authors [Aaltonen et al., 1987, 1988; Ansai et al., 2000; Carlsson et al., 1975; Grindefjord et al., 1995, 1996; Hirose et al., 1993; Holbrook, 1993; Holbrook et al., 1995; Li et al., 2000; Litt et al., 1995; Mattos-Graner et al., 2001; Radford et al., 2001; Raitio et al., 1995; Reisine et al., 1994; Rodriguez Miro et al., 1989; Roeters et al., 1995; Saemundsson et al., 1992; Shi et al., 2003; Twetman et al., 1994; Twetman and Petersson, 1996; Wendt et al., 1996; Seki et al., 2003b] were contacted for data on the subset of caries-free children at baseline and for data on children tested mutans streptococci-positive at baseline. Contacting the authors allowed for the inclusion of two additional publications [Litt et al., 1995; Wendt et al., 1996], while reviewing references of relevant primary articles found no additional study. Therefore, a total of 9 studies were available for this review. The median (25th to 75th centile range) number of participants was 88 (39–183).

Study Description

Study details are shown in tables 1 and 2.

Participants

Some studies reported on children of low socio-economic status [Litt et al., 1995; O'Sullivan and Thibodeau, 1996], on such living in suburbs [Alaluusua and Renkonen, 1983], on children attending kindergarten [Twetman et al., 1994] or Head Start classes [O'Sullivan and Thibodeau, 1996] and on children with recurrent otitis media and other upper respiratory tract infections mixed with randomly selected children of regular check-ups [Tenovuo et al., 1990]. Other reports studied children living in areas without water fluoridation [Alaluusua and Renkonen, 1983; Pienihäkkinen and Jokela, 2002; Seki et al., 2003a; Tenovuo et al., 1990; Twetman et al., 1994] or children using fluoridated dentifrices [Twetman et al., 1994].

Table 2. Quality assessment of included studies along with the number of extracted cross-tabulations and caries prevalence of study

Author year	Quality items[a]	Participants without mutans streptococci at baseline		Participants with mutans streptococci at baseline		Caries prevalence %
		development of caries during follow-up	no development of caries during follow-up	development of caries during follow-up	no development of caries during follow-up	
Tenovuo et al. [1990]	4 ,5, 9, 13–16	4	12	7	1	45.8
Alaluusua and Renkonen [1983]	1,4–6, 9, 11, 14, 15, 17	11	23	5	0	41
O'Sullivan and Thibodeau [1996]	3–5, 8–11, 14–16	10	20	24	34	38.6
Pienihäkkinen and Jokela [2002]	1, 4–6, 9–11, 13–16	15	133	22	36	18
Litt et al. [1995]	4–6, 9, 11, 14–16	10	30	96	47	37.3
Twetman et al. [1994]	1, 4–6, 9, 11, 12, 14–16	41	95			30.1
Rodriguez Miro et al. [1989]	1, 4, 6, 9, 14, 16	13	104			11.1
Wendt et al. [1996]	1, 2, 4–6, 9, 11, 14–16	3	23	21	63	22
Seki et al. [2003a]	1, 4–6, 9, 11, 14–16	saliva 8 plaque 6	52 55	4 5	6 4	17 17

[a] Numbers correspond to the quality list provided in the 'Appendix'.

Plaque and Mixed Testing

Results on plaque tests alone were available in three studies [Pienihäkkinen and Jokela, 2002; Seki et al., 2003a; Tenovuo et al., 1990]. In one study mixing plaque and saliva test results [Alaluusua and Renkonen, 1983] explorers were used to remove plaque from labioapproximal surfaces of maxillary incisors and a child was classified as positive if mutans streptococci were detected either in plaque or saliva, respectively. Allocation to risk groups in studies with plaque tests [Alaluusua and Renkonen, 1983; Pienihäkkinen and Jokela, 2002; Tenovuo et al., 1990] was executed by evaluating growth (or absence of growth) of bacterial colonies. One study [Seki et al., 2003a] counted plaque scores in colony-forming units (CFU) according to the manufacturer's chart.

Saliva Testing

All other studies used either unstimulated [Litt et al., 1995; O'Sullivan and Thibodeau, 1996; Rodriguez Miro et al., 1989; Wendt et al., 1996] or stimulated saliva samples [Seki et al., 2003a; Twetman et al., 1994]. CFU counting was applied in 3 studies [Litt et al., 1995; O'Sullivan and Thibodeau, 1996] and classification was low risk (0 CFU), moderate risk (1–50 CFU) or high risk (>50 CFU). In one study using the Dentocult-SM Strip mutans method the scores of 0 or 1 were considered as low risk and scores of 2 and 3 as high risk [Seki et al., 2003a]. One saliva study [Wendt et al., 1996] assessed caries risk depending on growth or absence of growth of mutans streptococci in bacterial cultures. We considered 0 CFU or score 0 and 1 in the Dentocult-SM Strip mutans method as test negative and else as test positive.

Children were examined by one [Twetman et al., 1994; Wendt et al., 1996] or several dentists [Litt et al., 1995; O'Sullivan and Thibodeau, 1996; Pienihäkkinen and Jokela, 2002; Seki et al., 2003a]. In all studies clinical examinations were carried out using a mirror (with magnification [Pienihäkkinen and Jokela, 2002]) and an explorer. All studies reported dmfs indices. X-rays were used in one study [Wendt et al., 1996] as was fibre-optic transil-

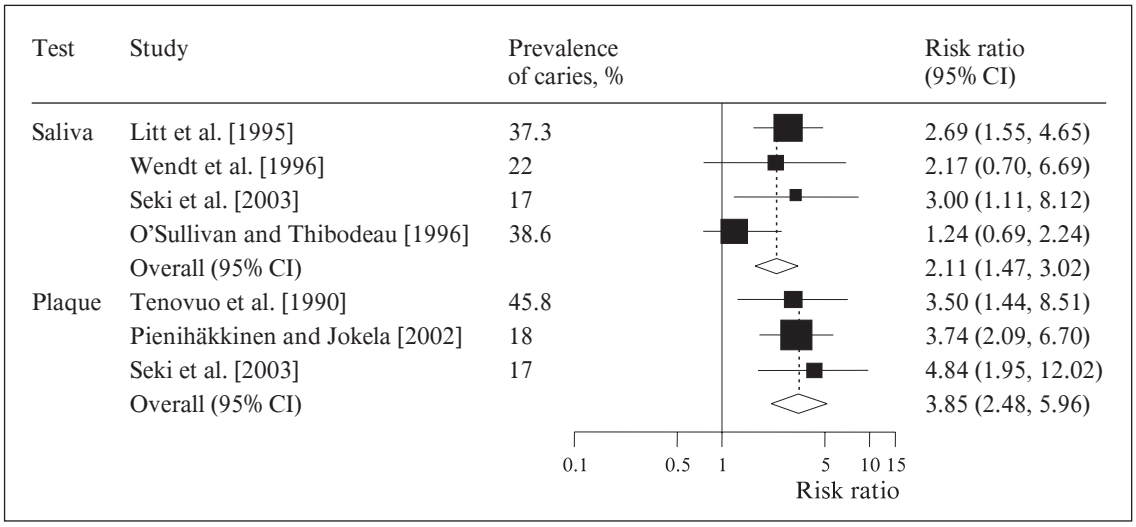

Fig. 2. Forest plot showing the relative risks for caries of individual studies stratified for articles reporting on the plaque test and the saliva test along with the prevalence of caries of each study. The black boxes indicate the point estimate of each study, the size of the box indicates the number of patients enrolled and the horizontal lines indicate 95% confidence intervals. The diamonds represent the pooled relative risk (and 95% confidence boundaries) for the two subgroups. One study [Alaluusua and Renkonen, 1983] was omitted from these analyses, because it only reported results for mixed saliva and plaque testing. The relative risk of that study was 3.09 (1.90, 5.03).

lumination [Pienihäkkinen and Jokela, 2002]. One study also included initial carious lesions [Wendt et al., 1996] and only data on children at risk were used. Caries diagnosis was performed on dry teeth in 2 studies [Pienihäkkinen and Jokela, 2002; Twetman et al., 1994].

Adjustment for Potential Confounding

Some studies adjusted for between 1 and 10 confounding factors (table 1), but no study adjusted for all the recommended confounders.

Methodological Quality

All reports investigated cohorts. In 8 studies detailed inclusion criteria were stated [Alaluusua and Renkonen, 1983; Litt et al., 1995; O'Sullivan and Thibodeau, 1996; Pienihäkkinen and Jokela, 2002; Seki et al., 2003b; Tenovuo et al., 1990; Twetman et al., 1994; Wendt et al., 1996]. Seven reports documented patients lost to follow-up [Alaluusua and Renkonen, 1983; Litt et al., 1995; O'Sullivan and Thibodeau, 1996; Pienihäkkinen and Jokela, 2002; Seki et al., 2003b; Twetman et al., 1994; Wendt et al., 1996] and 1 provided characteristics of those lost to follow-up [Twetman et al., 1994]. Blinding of persons assessing the outcome was stated in 2 studies [Pienihäkkinen and Jokela, 2002; Tenovuo et al., 1990] and detailed description on methods to assess outcomes was given in 8 reports [Alaluusua and Renkonen, 1983; Litt et al., 1995; O'Sullivan and Thibodeau, 1996; Pienihäkkinen and Jokela, 2002; Seki et al., 2003b; Tenovuo et al., 1990; Twetman et al., 1994; Wendt et al., 1996]. Details on all assessed items are provided in table 2.

Results

Pooled Relative Risks

Figure 2 shows the relative risks of studies reporting on saliva and plaque mutans streptococci tests. From one article [Seki et al., 2003b] we only included caries-free children at baseline. Two other articles [Rodriguez Miro et al., 1989; Twetman et al., 1994] could not be included in our meta-analysis because only the low risk groups consisted of caries-free children at baseline and stratified results were not obtainable. In another study [Pienihäkkinen and Jokela, 2002], we only included the group that received routine prevention.

The pooled relative risk (95% confidence interval) of 3 studies [Pienihäkkinen and Jokela, 2002; Seki et al., 2003b; Tenovuo et al., 1990] on 300 subjects detecting mutans streptococci in plaque from different tooth sur-

Table 3. Sensitivity (95% confidence interval), specificity (95% confidence interval) and predictive values of individual studies stratified for articles reporting on the plaque test and the saliva test along with the prevalence of caries in each study

Test	Study	Prevalence	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Saliva	Litt et al. [1995]	37.3	91 (83.3–95.4)	39 (28.1–50.8)	67	75
	Wendt et al. [1996]	22	88 (67.6–97.3)	27 (17.8–37.4)	25	88
	Seki et al. [2003]	17	33 (9.9–65.1)	90 (78.8–96.1)	40	87
	O'Sullivan and Thibodeau [1996]	38.6	71 (52.5–84.9)	37 (24.3–51.3)	41	67
Plaque	Tenovuo et al. [1990]	45.8	64 (30.8–89.1)	92 (64.0–99.8)	88	75
	Pienihäkkinen and Jokela [2002]	18	59 (42.1–75.3)	79 (71.8–84.6)	38	90
	Seki et al. [2003a]	17	45 (16.8–76.6)	93 (83.5–98.1)	55	90

One study [Alaluusua and Renkonen, 1983] was omitted, because it only reported results for mixed saliva and plaque testing.

faces was 3.85 (2.48–5.96). The pooled relative risk (95% confidence interval) of 4 studies [Litt et al., 1995; O'Sullivan and Thibodeau, 1996; Seki et al., 2003b; Wendt et al., 1996] on 451 subjects detecting mutans streptococci in stimulated or unstimulated saliva was 2.11 (1.47–3.02). The relative risk of 1 study [Alaluusua and Renkonen, 1983] reporting on mixed testing was 3.09 (1.90–5.03).

Accuracy Data

The sensitivity of the mutans streptococci test ranged between 33 and 91% for the saliva tests and between 45 and 64% for the plaque test, respectively. The corresponding values for the specificity were between 27 and 90% for the saliva test and 79 and 93% for the plaque test, respectively. Values for each single study and the corresponding predictive values are shown in table 3.

Discussion

This systematic review shows that the detection of mutans streptococci in saliva or plaque of 2- to 5-year-old children is associated with an increased risk for developing caries. The extent of the association, however, remains uncertain for several reasons. First, most of the studies either did not report statistical adjustment for potential confounders at all or only used an incomplete list, compared with that presented above. Incomplete adjustment for potential confounding can lead to biased results, but unfortunately, the direction of this bias cannot be estimated. Furthermore, the low number of relevant studies, the small sample sizes, and differences in methods of testing and outcome assessment applied limit the interpretation of the results. Finally, there was substantial variability in the method used to detect mutans streptococci in plaque or saliva samples. Plaque samples were taken from different sites in the oral cavity (approximal areas of deciduous molars or of maxillary incisors), which are not equally prone to bacterial colonisation [Bratthall et al., 1995; Macpherson et al., 1990]. Samples of stimulated or unstimulated saliva were used although the two types of oral fluid harbour different numbers and species of bacteria. Various sample dilutions and a variety of incubation procedures with differing sensitivity were used in the reviewed studies.

Nevertheless, the present systematic review showed consistent findings among studies assessing mutans streptococci in plaque (heterogeneity $\chi^2 = 0.23$) and saliva (heterogeneity $\chi^2 = 0.86$), respectively, which is partly reassuring. One study [Seki et al., 2003b] allowed a direct comparison of saliva and plaque testing and also confirmed the results reported here. Sensitivities and specificities varied both within and between studies reporting on saliva and plaque tests. However, results of plaque tests were more consistent and showed higher values compared to saliva tests. This may indicate that, if tested positive, the patient may develop more often caries.

Clinical Interpretation

Due to several methodological shortcomings of the primary studies, recommendations for the general usage of the mutans streptococci test as a risk assessment tool in preschool children cannot yet be justified. However, if

future well-designed studies confirm the hypothesis that mutans streptococci-positive children have an increased caries risk, intensified prophylactic measures in children with an increased risk of caries with more specific and costly interventions, such as fluoridation liquids, antimicrobial agents like chlorhexidine and repeated intensified dental check-ups seem advisable.

Conclusion

The presence of mutans streptococci both in plaque or saliva of young caries-free children appears to be associated with a considerable increase of caries risk. However, further well-designed studies are required to confirm the results reported in this review. If these studies corroborate our findings an intensified caries prevention programme seems advisable in mutans streptococci-positive yet caries-free children.

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Appendix

Validity checklist for observational studies

External validity	
1	Subjects invited to participate representative of entire population
2	Proportion of invited subjects who agreed
3	Demonstration of the distribution of confounding factors in study population and entire population
Internal validity	
4	Clear description of objective of the study
5	Definition of inclusion criteria
6	All participants recruited from the same population
7	Registration of confounding factors in each group
8	All confounding factors considered
9	Explanation of prognostic test
10	Registration of prophylactic interventions during follow-up
11	Registration of loss to follow-up
12	Characteristics of participants lost to follow-up
13	Blinding of persons who assess outcome
14	Definition of outcome
15	Accurate methods to assess outcome
Data description and analysis	
16	Appropriate statistical methods
17	The way missing values were dealt with
18	The distribution of baseline characteristics
19	Adjustment for confounding

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