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Caries-associated micro-organisms in infants from different socio-economic backgrounds in Scotland

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Abstract

Objectives: The aims of this study were: (1) to compare the frequency of isolation of mutans streptococci, (*Streptococcus mutans*, *Steptococcus sobrinus*), lactobacilli and yeasts (caries-associated micro-organisms) in the saliva of 1-year-old infants with and without dental caries; and (2) to determine if socio-economic background influenced the frequency isolation of bacteria and caries status.

Methods: 1393 1-year-old consented infants, who comprised 70.3% of children born in Dundee during a 1 year period, had saliva samples taken (tongue-loop method) for microbiological culture and were examined for dental caries (d_1-threshold: enamel and dentine diagnostic threshold). Thirty-nine infants were diagnosed with caries and the frequencies of isolation of caries-associated micro-organisms (and absolute microbial counts) were compared with infants who were caries-free. In addition, associations were sought between the infants' socio-economic background, the frequency of isolation of caries-associated micro-organisms and caries status.

Results: Streptococcus mutans, lactobacilli and yeasts were isolated more frequently from those infants with caries compared to those who were caries-free (*S. mutans*: 29.7 vs 9.8%, $P = 0.0008$; lactobacilli: 15.4 vs 4.3%, $P = 0.0073$; yeasts: 23.7 vs 10.4%, $P = 0.0016$—Fisher's exact test). There were no significant differences between the isolation frequencies of *S. sobrinus* (2.7 vs 1.3%, $P = 0.39$) from those with and without caries. Significantly, more infants living in areas of high deprivation had caries compared to those from more affluent areas (DEPCAT 6 and 7 vs 1–5: 3.6 vs 1.9%, $P = 0.049$), but, apart from yeasts, socio-economic background was not significantly associated with the isolation frequencies of any of the caries-associated micro-organisms.

Conclusions: In infants as young as 1 year of age, salivary *S. mutans*, lactobacilli and yeasts but not *S. sobrinus* were isolated significantly more frequently from those with caries compared to those who were caries-free. Apart from yeasts, socio-economic background did not influence the frequency of isolation of caries-associated micro-organisms. However, infants living in areas of highest deprivation had significantly higher frequencies of caries compared to those from more affluent areas.

Keywords: Mutans streptococci; Infants; Caries; Socio-economic

1. Introduction

There continues to be stark inequalities in the oral health of children with more than half the untreated tooth decay found in just 10% of 5 year-olds [1]. Identification of such children before caries has occurred would allow targeting of preventative care. A key putative marker is caries-associated micro-organisms and indeed many studies have reported correlations between mutans streptococci and caries status in adolescents (for key papers see [2–4]), but few [5–7] have looked for associations in infants, particularly as young as 1 year of age.

In addition, there is little information about relations between socio-economic status and caries experience in infants and, of those, no clear conclusion can be made [8–12]. There also appears no report in the literature examining the relationship between the frequency of isolation of mutans streptococci, lactobacilli and yeasts (caries-associated micro-organisms) and socio-economic status in 1-year-old infants.

As part of a 4 year longitudinal project with the aim of identifying markers for caries in pre-school infants, oral microbiological sampling and dental examinations were carried out on 1393, 1-year-old infants. Thirty-nine of

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these infants already had caries (when diagnosed at the d_1-threshold: enamel and dentine diagnostic threshold). The aims of this study therefore, were: (1) to compare the frequency isolation of caries-associated micro-organisms in infants with and without dental caries; and (2) to determine if socio-economic background was associated with microbial carriage and caries status.

2. Materials and methods

Health visitors carried out the oral microbiological sampling and a research dentist (HB) performed the caries examination. These data were collected in a 'field setting', viz. in the infants' homes, at health centres and at nurseries.

2.1. Subjects

The parents/guardians of all children (1981) born in Dundee, Scotland, during one calendar year (April 1993–March 1994) were approached for permission to allow their children to have microbiological samples taken, to be dentally examined and for other information to be collected including socio-demographic data. From a total of 1703 consented infants, salivary samples were taken from 1436, and 1419 were dentally examined. Salivary microbial and caries data were recorded for 1393 infants, who make up the group reported in this paper. Eighty-nine per cent of microbiological samples and dental examinations were carried out within a window of 3 months before, or 3 months after, the infant's first birthday.

The protocol for this non-interventionist designed caries-risk assessment study was approved by Tayside Committee on Medical Research Ethics.

2.2. Measure of relative deprivation or affluence

This is quantified in Scotland according to the method described by Carstairs and Morris [13]. In summary, scores (DEPCAT categories) are derived from 1991 Census data for populations in postcode sectors by combining the following variables: overcrowding, male unemployment, low social class and whether or not the household owns a car. The score is therefore a measure of a particular sector's socio-economic status relative to the average for Scotland, a score of DEPCAT 1 being the most affluent and 7 the most deprived. Such data can be obtained from the Public Health Research Unit, University of Glasgow, G12 8RZ (Carstairs Scores for Scottish Postcode Sectors from the 1991 Census, ed.: Philip McLoone).

In Dundee, only one child was born during the year in the DEPCAT 7 area and this single observation was included in DEPCAT 6.

2.3. Oral microbiological sampling and processing

The tongue-loop method [14] was used to collect the oral microbiological samples. Each sample was agitated into a vial containing 1 ml of LAB M Fastidious anaerobe broth (LAB M, Bury, England BL9 6AU), placed in a polystyrene block in a Combi Cold Carrier®, which was kept cool in a GIO'STYLE® cold picnic box (Jencons [Scientific] Ltd, Leighton Buzzard, England LU7 8UA) before transportation to a laboratory for microbiological processing.

Caries-associated micro-organisms were cultured and characterised as described by Beighton et al. [15]. In summary, samples were dispersed by vortexing for 10 s and mutans streptococci (*Streptococcus mutans and Streptococcus sobrinus*) were cultured on mitis salivarius agar plus 15% sucrose and 0.2 units per ml bacitracin, lactobacilli on Rogosa agar and yeasts on Sabourand Dextrose Agar (all media from Oxoid, Unipath Ltd, Basingstoke, UK). Plating-out was carried out within 4–8 h of collection. The mutans streptococci and the lactobacilli were incubated anaerobically and the yeasts were cultured aerobically for 3 days at 37°C.

After incubation, colonies with a characteristic morphology (*S. mutans*—raspberry-shaped and embedded into agar; *S. sobrinus*—again raspberry-shaped and embedded into agar but surrounded by a 'halo'; yeasts—white, flat and matt with a creamy consistency and a distinctive malodour; lactobacilli—the only colonies recovered on Rogosa agar) were counted, tested for catalase and Gram-stained. Confirmative identification of mutans streptococci was carried on a representative sample of presumptive isolates, based on a short set of biochemical and fermentation tests [16]. The lowest detection level for each of these taxa were 10^3 colony forming units per ml of saliva.

2.4. Caries diagnosis

A calibrated examiner (HB) carried out dental examinations on all the infants. Caries was diagnosed at the d_1 threshold according to the method described by Fyffe [17] based on a visual classification described by the World Health Organisation [18]. A pen torch (pen light) aided this 'field' examination.

2.5. Statistical analysis

Data were analysed using the SPSS® software package. The relationship of isolation frequencies of each of the bacterial taxa to caries status were examined using Fisher's exact test.

Microbiological data were also expressed as absolute counts of bacteria and Mann–Whitney U tests were used to look for differences between infants with caries and those who were caries-free. Receiver Operating Characteristic curve (ROC) analysis [19] was used to compare the relative efficacy of microbial counts as a diagnostic test for caries.

Associations between the degree of relative affluence or deprivation, as measured by DEPCAT, and caries and recovery of caries-associated micro-organisms were examined using the Mantel–Haenszel test for linear association. Because the majority of infants in Dundee live in areas of

Table 1
Caries-associated micro-organisms (% frequency isolation) in infants with and without caries

	Infants with caries	Caries-free	All infants	Fisher's exact test	Mann–Whitney[a] U test
Mutans streptococci	32.4	10.2	10.8	0.0003	0.0001
S. mutans	29.7	9.8	10.3	0.0008	< 0.0001
S. sobrinus	2.7	1.3	1.3	0.39	0.46
Lactobacilli	15.4	4.3	4.6	0.0073	0.001
Yeasts	23.7	10.4	10.6	0.0016	0.0066

[a] To look for differences in absolute microbial counts between those infants with caries and caries-free infants.

high deprivation, data from DEPCAT 1 and 2 were pooled for comparisons, as were the data from DEPCAT 6 and 7.

3. Results

Thirty-nine from a total of 1419 infants had caries. These 39 infants suffered from 116 decayed surfaces (94 enamel and 22 dentinal). Seventy-eight of the affected surfaces were on the upper incisors (only the upper and lower incisor teeth had erupted in the majority of children).

Isolation frequencies of caries-associated micro-organisms in infants with caries and those who were caries-free are shown in Table 1. Mutans streptococci were isolated significantly more frequently from those infants with caries compared to those who were caries-free. The mutans streptococci were comprised almost entirely of *S. mutans*. Similarly, lactobacilli were isolated significantly more frequently from those infants with caries compared to those with no caries, as were yeasts. This was in contrast to *S. sobrinus* where there were no significant differences between the groups. Regardless of caries status, mutans streptococci, *S. mutans*, *S. sobrinus*, lactobacilli and yeasts were isolated from 10.8, 10.3, 1.3, 4.6 and 10.6%, respectively.

When absolute microbial counts were analysed, similar differences were found between those infants with caries and those who were caries-free (Table 1). Receiver Operator Characteristic curves (Fig. 1) showed that absolute microbial counts were not a good surrogate diagnostic test for caries.

The degree of relative affluence or deprivation did not significantly influence the proportion of infants who harboured mutans streptococci and lactobacilli except for yeasts, which were associated with greater deprivation (Table 2) This association of yeasts with deprivation was maintained when infants with $d_1t > 0$ are excluded from the analysis.

Those infants living in areas of greatest deprivation had significantly higher mean caries (DEPCAT 6 and 7 vs 1–5: 3.6 vs 1.9%, $P = 0.049$).

4. Discussion

This study has demonstrated that in Dundee, Scotland, 1-year-old infants with caries have higher isolation frequencies and higher counts of *S. mutans*, lactobacilli and yeasts but not *S. sobrinus* compared to those who were clinically caries-free. Similar findings have been published by Grindefjord et al. [20] who reported that mutans streptococci and lactobacilli were significantly associated with caries in 2.5-year-old children (over half were categorised as 'children with immigrant background') living in Stockholm. In addition, numbers of mutans streptococci have been shown to be significantly correlated with caries prevalence in 365 1- and 2-year-old Japanese infants [7]. These results would appear to contrast with those reported by Matee et al. [21] who found there were no differences in the isolation frequencies

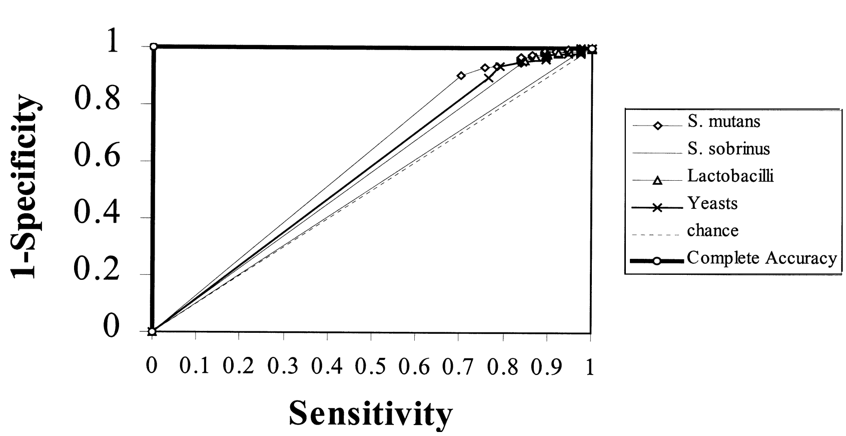

Fig. 1. Receiver–Operator Characteristic (ROC) curves for microbial counts.

Table 2
Associations between (1) caries and (2) the recovery of caries-associated micro-organisms and DEPCAT

	DEPCAT					*P* (all infants[a])	*P* ($d_1t > 0$ excluded[b])
	1 and 2	3	4	5	6 and 7		
No. of infants	318	99	188	66	722		
% dmft > 0	0.6	1.9	2.6	6.2	3.6	0.005	–
% Mutans streptococci	8.6	8.7	13.6	12.1	11.1	0.26	0.43
% *S. mutans*	8.0	7.8	12.6	12.1	10.9	0.14	0.23
% *S. sobrinus*	1.2	1.0	1.6	0	1.4	0.87	0.95
% Lactobacilli	2.7	6.8	3.6	4.4	5.1	0.15	0.10
% Yeasts	7.8	10.8	9.9	9.2	12.3	0.039	0.041

[a] Mantel–Haenszel test probabilities for all infants.
[b] Mantel–Haenszel test probabilities if infants with $d_1t > 0$ are excluded.

of *S. mutans* (plaque samples) between caries-active and caries-free 1–3.5-year-old infants from regions of Tanzania with high and low prevalences of caries. It would appear, however, that although there is a correlation between caries and mutans streptococci in industrialised countries, this may not be generalisable for young children in non-industrialised countries such as those on the African continent [22].

Isolation rates for mutans streptococci in infants vary dramatically between studies. In this study, 10.8% of infants harboured mutans streptococci which is comparable to that reported by Grindefjord [23] who recovered mutans streptoccoci from 6% of a group of 1095 1-year-olds. These results are in contrast to other studies, in similar age groups, which have reported isolation frequencies varying from 43% [24] to circa 90% in Canadian children [25]. A possible explanation for such variation could be methodological differences arising from sampling and culture. However, it has been reported [22] that the viable counts of mutans streptococci obtained using the spatula method, the tongue-loop method, commercial dip-slide methods and others are all significantly correlated with the counts obtained using conventional paraffin-stimulated saliva cultured on selective media. It would therefore appear that methodological differences alone would not account for such variation. Another possible explanation is the difference in sugar consumption between the various demographic groups of children, although the relationship between *S. mutans* and diet in the population is far from clear [4,26,27], as many studies have been carried out under abnormal or extreme conditions [28].

Mutans streptococci were recovered from only one-third of infants with caries. It maybe that seeding of bacteria from d_1 lesions into saliva is not very effective or alternatively the numbers of mutans streptococci were less than the lowest detection level (10^3 colony forming units per ml of saliva).

Few studies have reported the frequency of lactobacilli in pre-school children. In our study, lactobacilli was recovered from 4.6% of infants which is less than but comparable to that reported by Roeters [29] (11.5%) who sampled 252, 2-year-olds at baseline in a 3-year cohort study and 13.1% in 36–47-month-old Caucasian children in a study [30] which compared caries prevalence and caries-associated micro-organisms in Caucasian and Afro-Caribbean children. This is in contrast to the results reported by Kohler et al. [31] who found that approximately 40% of 3-year-old children carried lactobacilli in their saliva.

Streptococcus sobrinus was isolated from only 1.3% of infants. This was not unexpected as this species was only recovered from 7% of 5–8-year-old Hampshire schoolchildren implying a low prevalence in the UK [32].

In contrast to the smaller sample sizes employed in many studies, in the present study 1400 children were examined thereby minimising the effect of individual variation. In addition, microbiological sampling and dental examinations were carried out within 3 months of the infant's first birthday, reducing the effect of age on the increased acquisition of oral bacteria [33] and caries increments. Few studies have reported the caries prevalence in 1-year-old children although recently two major studies [11,12] have been published. Four per cent of children from the UK aged 1.5–2.5 and 6.4% of 1-year-old children from the US had caries experience at the d_3 (caries into dentine) level. The present study found that 2.7% of infants were suffering from dental caries, which is within the same range as that reported by Wendt et al. [34] who studied 632, 1-year-old Swedish children (again using the d_3 diagnostic threshold).

In the present study, caries was diagnosed at the d_1 level. Ideally, in order to examine caries at this threshold, teeth should be dried before examination. It was decided to carry out the caries examinations without an airline to dry the teeth as it was anticipated that this could lead to infant distress, reducing the acceptability of the examination. Performing the dental examination in a 'field setting' allowed us to access more children.

Seventy eight per cent of carious surfaces diagnosed in this study affected the upper incisor teeth which was not surprising as, at 1 year of age, generally only the upper and lower incisor teeth are fully erupted with the first lower deciduous molars partially erupted. Grindefjord et al. [20] reported that 72% of carious lesions in their study were localised to the maxillary incisors of 2.5-year-old children. They suggested that this might partly be explained by a higher intake of sugar-containing beverages at night. This poses the vexing question as to whether or not a significant proportion of caries observed in under 2-year-olds is in fact nursing caries (early childhood caries—ECC [35]). It should be pointed out, however, there is much confusion surrounding the prevalence and epidemiology of ECC, some questioning as to whether indeed this is a distinct entity only distinguished by the generalised distribution of the lesions.

Krasse [36] paradoxically referred to those children with more than 10^6 *S. mutans*/ml of saliva and at particular risk from dental caries, as 'millionaires'. This was based on a study of a large group of 645, 9–12-year-old children who showed that those with these high numbers of *S. mutans* and lactobacilli developed significantly more carious lesions after 1 and 2 years compared to children with low counts. We used ROC analysis [19], to determine if such a relationship could be found in 1-year-olds. Although microbial levels are related to caries status, they did not constitute a good screening test and this was not unexpected as a stark microbial threshold is probably a gross simplification of the carious process [37].

There do not appear to be any papers in the literature that have reported associations between socio-economic status and the recovery of caries-associated micro-organisms in 1-year-old infants. Apart from yeasts, this study was not able to demonstrate any such relationship. This was not unexpected as few teeth had erupted in 1-year-olds and, of those present, there had been insufficient time for the establishment of an amphibiont microflora.

However, it was found that those infants, even as young as 1 year of age, living in areas of high deprivation had a greater experience of caries compared to those from more affluent backgrounds. This supports the findings of a multi-stage random probability design study [11] analysing data derived from The National Diet and Nutrition Survey of UK children aged 1.5–4.5 years, which reported that caries was found to be most strongly related to receipt of income benefit, educational status of the mother and social class of the head of the household. Interestingly, this simple relationship was not found in 2-year-old children from the Riyadh region of Saudi Arabia [8], 1- and 2-year-olds from Goiânia-GO, Brazil [9] and school children living in the inner city are of Camden, London, UK [10] (in contrast to ethnicity in this latter study). Roeters [38] reported that the correlation between socio-economic background and caries becomes stronger with increasing age (as also reported in groups of older children in Riyadh and Goiânia). Despite a high proportion of our sample lived in areas of high deprivation (the Arizona preschool study [12] purposely oversampled children from low-income backgrounds and therefore were not able to look for such an association), the size of our group enabled us to demonstrate a relationship between socio-economic background and caries that could not have been shown in a smaller experimental group.

One of the key questions we aim to answer is whether those pre-school infants who harbour caries-associated micro-organisms but who do not have caries at 1 year of age are at increased risk from future caries compared to those who do not carry these microbial taxa. If such a relationship could be demonstrated, microbiological sampling of saliva to identify pre-school infants at risk of future caries offers a strategy for targeting preventive care at these infants. Analysis of longitudinal data will seek to answer this question.

In conclusion therefore, this study has shown that *S. mutans*, lactobacilli and yeasts were isolated significantly more frequently from the saliva of 1-year-old infants with caries compared to those who were caries-free. Socio-economic background did not influence the frequency of isolation of caries-associated micro-organisms apart from yeasts, although those infants living in areas of high deprivation had significantly more caries than those from more affluent areas.

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