# Risk factors for colonization of salivary lactobacilli and candida in children

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We analyzed the occurrence of salivary lactobacilli and candida in a sample of 166 children aged 1–4 years (mean, 2.5 years) in relation to possible risk factors for colonization of the microbes. The risk factors examined were sucking habits, feeding habits, symptoms of respiratory infections, and antibiotic therapy at the time of sampling. Lactobacilli occurred in 18% of the children's saliva, and candida in 24%. In the logistic modeling the variables most strongly associated with the occurrence of lactobacilli were pacifier-sucking (relative risk (RR), 2.9; 95% confidence interval (CI), 1.1–7.0; $P = 0.01$) and antibiotic therapy (RR, 4.6; CI, 1.2–16.9; $P = 0.01$). The association between positive candida tests and use of a pacifier was also significant (RR, 4.8; CI, 2.1–10.7; $P = 0.0001$). The results of the study suggest that the use of a pacifier increases the occurrence of both salivary lactobacilli and candida. It could therefore be a factor influencing caries susceptibility and activity in children. □ *Antibiotic therapy; caries activity; caries susceptibility; feeding habit; sucking habit*

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The frequency of salivary lactobacilli found during the first years of life varies. Lactobacilli were found in about half of the saliva samples in 1-year-old children in the survey by McCarthy et al. (1). Carlsson et al. (2), however, found that during the first 2 years of life salivary lactobacilli occurred only transiently and in very low numbers. Salivary candida has been found in 24% of premature infants, in 4% of full-term babies aged 4–5 days, and in about 30% of children between 3 and 12 years (3). McCarthy et al. (1) isolated candida from less than half of the infants at the age of 12 months.

Oral lactobacilli and yeasts increase as a result of an increase in the number of retentive sites in the oral cavity (4). Such retentive sites include prostheses (5, 6) and orthodontic appliances (7–9).

Children who use pacifiers have clinical thrush and positive mouth swabs for candida more frequently than those who do not (10, 11). The use of a pacifier is also suggested to increase the frequency of dental caries. Larsson (12) showed a statistical relationship between use of a pacifier and caries in 4-year-old children. Furthermore, it is generally known that sucking habits are connected with malocclusions and disturbances in dentofacial development (13–15).

We hypothesized that sucking habits, feeding habits, antibiotic therapy, and common respiratory infections in early childhood would affect the colonization of saliva with lactobacilli and candida, which are important microbes in the regulation of caries activity and susceptibility (16). To test this hypothesis, we analyzed the salivary microbial counts with practical clinical dip-slide tests and sucking habits and other possible risk factors in a population of healthy children.

## Materials and methods

The present study was a part of a large infection survey investigating the connection of pacifier sucking to susceptibility to otitis media infections. The size of the study population was determined on the basis of the prevalence of otitis media infections. One hundred and eighty-three children aged 1–4 years were entered in the study, which was conducted at 11 day-care centers in the town of Oulu, Finland. These day-care centers were chosen on the basis of their willingness to participate in the survey and the number of children they cared for. Seventeen of the children were subsequently excluded either because they neglected to return the questionnaire or because it proved impossible to obtain a proper sample of saliva. The final analysis was therefore performed on 166 children (88 boys and 78 girls).

The mean age of the children was 2.5 years (range, 0.7–4.3 years). Seventy-eight per cent had used a pacifier, and 6% had been thumb-suckers. Pacifier-sucking lasted on average 16.5 months (range, 0–42 months). At the time of sampling, 39% were still using a pacifier, and 4% were still sucking their thumb (Table 1).

The mean duration of breast-feeding was 4.9 months (range, 0–24 months), and 6% of the children had been breast-fed for more than a year. The duration of breast-feeding was defined as the time during which the child received any amount of breast milk regardless of supplementation with formula or other foods. About half of the children (54%) had used a nursing bottle at night for an average of 10.5 months (range, 0–40 months). Possible risk factors in the subjects at the time of sampling are shown in Table 1.

Table 1. Possible risk factors for positive salivary lactobacilli and candida tests in the whole study population ($n$ = 166) and in children <2 years ($n$ = 48) and in children ≥2 years ($n$ = 118) at the time of sampling

| Variables | All | | Children <2 years | | Children ≥2 years | |
|---|---|---|---|---|---|---|
| | % | (n) | % | (n) | % | (n) |
| Pacifier-sucking | 39 | (65) | 73 | (35) | 25 | (30) |
| Thumb-sucking | 4 | (6) | 4 | (2) | 3 | (4) |
| Breast-feeding | 2 | (3) | 6 | (3) | 0 | (0) |
| Nursing bottle at night | 28 | (46) | 54 | (26) | 17 | (20) |
| Symptoms of respiratory infections | 63 | (104) | 69 | (33) | 60 | (71) |
| Antibiotic therapy | 8 | (13) | 19 | (9) | 3 | (4) |

Informed consent for participation in this study was obtained from the parents, and the study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Oulu.

*Questionnaire*

Information on the sucking habits and other background information was collected from a questionnaire filled in by the parents. The questionnaire included questions about pacifier- and thumb-sucking, breast-feeding, bottle-feeding at night, and symptoms and signs of respiratory infections during the last 2 weeks. The symptoms included rhinitis, cough, fever >38 °C, and conjunctivitis. The symptoms were classified as positive when at least one of the symptoms existed. Information about antibiotic therapy at the time of sampling was obtained from the day-care center.

*Microbial sampling*

Saliva samples for lactobacilli and candida counts were taken from unstimulated saliva sublingually with cotton swabs. The swabs were immediately cultivated over one side of Dentocult® LB and Oricult® N slides (Orion Diagnostica, Espoo, Finland) (17). The samples were incubated at 37 °C for 2 days (Oricult N) or 4 days (Dentocult LB), after which the occurrence of microbes was determined. Only the presence or absence of colonies was recorded, the cut-off value being 1 colony-forming unit (CFU).

*Statistical analysis*

The associations between the occurrence of microbes and the possible risk factors were evaluated by both univariate analysis and logistic multivariate regression modeling. Direct univariate associations were studied by cross-tabulation of the dichotomous variables. Statistical differences were tested using the chi-square test. As the present study was designed as a cohort study defined by exposure, possible risk factors were analyzed by calculating their relative risks (RR) and 95% confidence intervals (CI). To control for age, the analyses were also performed separately in two age groups: children younger than 2 years and children 2 years or older.

Stepwise logistic multivariate modeling was used to control for confounding variables. The occurrence of both microbes was used as dependent variables. The covariates were the use of a pacifier, thumb-sucking, the use of a nursing bottle at night, breast-feeding, symptoms of respiratory infections, and antibiotic therapy. Age was also included in the multivariate analysis. The method used in logistic regression analysis was forward stepwise as described in the manual of SPSSWin, version 6.0 (18).

The data for all subjects on whom information was available were used in the univariate analysis, whereas logistic multivariate modeling included only the subjects for whom data about all the variables in the model were available.

The statistical analyses were performed using SPSSWin, version 6.0.

## Results

In the whole study population lactobacilli were found in 18% of the cultures of the children's saliva, and candida in 24%. In the younger age group (<2 years) salivary lactobacilli occurred in 25% and candida in 33%. In the older group (≥2 years) the frequencies were 15% for lactobacilli and 19% for candida.

The use of a pacifier was significantly associated with a positive salivary lactobacilli test (RR, 3.1; CI, 1.5–6.2) (Table 2) and candida test (RR, 2.7; CI, 1.5–4.9) (Table 3), as were nursing bottle at night and antibiotic therapy. Thumb-sucking, breast-feeding, and symptoms of respiratory infections were not related to the occurrence of either microbe (Tables 2 and 3).

When children younger and older than 2 years were considered separately, in the younger group (<2 years) symptoms of respiratory infections were the only variable significantly related to the occurrence of both microbes (Tables 2 and 3). In the older group positive

Table 2. Sucking and feeding habits, symptoms of respiratory infections, and antibiotic therapy in children with positive or negative lactobacilli tests, with relative risks (RR) and 95% confidence intervals (CI)

| | Lactobacilli test | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | All ($n$ = 166) | | | Children <2 years ($n$ = 48) | | | Children ≥2 years ($n$ = 118) | | |
| Variables | Positive, $n$ = 30, % ($n$) | Negative, $n$ = 136, % ($n$) | RR (CI) | Positive, $n$ = 12, % ($n$) | Negative, $n$ = 36, % ($n$) | RR (CI) | Positive, $n$ = 18, % ($n$) | Negative, $n$ = 100, % ($n$) | RR (CI) |
| Pacifier-sucking | 67 (20) | 33 (45) | 3.1 (1.5–6.2)*** | 83 (10) | 69 (25) | 1.8 (0.4–7.3) | 56 (10) | 20 (20) | 3.6 (1.5–8.4)** |
| Thumb-sucking | 0 | 4 (6) | 0.3 (0.02–5.8) | 0 | 6 (2) | 0.5 (0.02–11.0) | 0 | 4 (4) | 0.5 (0.03–10.4) |
| Breast-feeding | 0 | 2 (3) | 0.6 (0.03–11.6) | 0 | 8 (3) | 0.4 (0.02–7.3) | 0 | 0 | — |
| Nursing bottle at night | 47 (14) | 24 (32) | 2.2 (1.2–4.2)** | 67 (8) | 50 (18) | 1.6 (0.5–4.8) | 33 (6) | 14 (14) | 2.4 (1.0–5.7)* |
| Symptoms of respiratory infections | 67 (20) | 62 (84) | 1.1 (0.5–2.3) | 92 (11) | 61 (22) | 5.0 (0.7–35.2)* | 50 (9) | 62 (62) | 0.6 (0.2–1.5) |
| Antibiotic therapy | 23 (7) | 4 (6) | 3.5 (1.8–6.6)*** | 33 (4) | 14 (5) | 2.1 (0.8–5.4) | 17 (3) | 1 (1) | 5.7 (2.7–11.9)** |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Sucking and feeding habits, symptoms of respiratory infections, and antibiotic therapy in children with positive or negative candida tests, with relative risks (RR) and 95% confidence intervals (CI)

| | Candida test | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | All ($n$ = 166) | | | Children <2 years ($n$ = 48) | | | Children ≥2 years ($n$ = 118) | | |
| Variables | Positive, $n$ = 39, % ($n$) | Negative, $n$ = 127 % ($n$) | RR (CI) | Positive, $n$ = 16, % ($n$) | Negative, $n$ = 32 % ($n$) | RR (CI) | Positive, $n$ = 23, % ($n$) | Negative, $n$ = 95 % ($n$) | RR (CI) |
| Pacifier-sucking | 64 (25) | 32 (40) | 2.7 (1.5–4.9)*** | 88 (14) | 66 (21) | 2.6 (0.6–9.9) | 48 (11) | 20 (19) | 2.6 (1.3–5.4)** |
| Thumb-sucking | 5 (2) | 3 (4) | 1.4 (0.4–4.5) | 0 | 6 (2) | 0.3 (0.02–7.6) | 9 (2) | 2 (2) | 2.6 (0.9–7.7) |
| Breast-feeding | 3 (1) | 2 (2) | 1.4 (0.2–7.5) | 6 (1) | 6 (2) | 1.0 (0.1–5.2) | 0 | 0 | — |
| Nursing bottle at night | 41 (16) | 24 (30) | 1.8 (1.0–3.1)* | 69 (11) | 47 (15) | 1.8 (0.7–4.5) | 22 (5) | 16 (15) | 1.3 (0.5–3.2) |
| Symptoms of respiratory infections | 67 (26) | 61 (78) | 1.1 (0.6–2.1) | 88 (14) | 59 (19) | 3.1 (0.8–12.2)* | 52 (12) | 62 (59) | 0.7 (0.3–1.4) |
| Antibiotic therapy | 16 (69) | 6 (7) | 2.1 (1.1–4.2)* | 27 (4) | 16 (5) | 1.5 (0.6–3.7) | 9 (2) | 2 (2) | 2.7 (0.9–7.7) |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

candida tests were associated only with pacifier-sucking (Table 3).

The variables most strongly associated with the occurrence of lactobacilli according to multivariate logistic regression analysis were pacifier-sucking (RR, 2.9; CI, 1.1–7.0; $P = 0.01$) and antibiotic therapy (RR, 4.6; CI, 1.2–16.9; $P = 0.01$). When a positive candida test was the dependent variable, pacifier-sucking was the only significant risk factor (RR, 4.8; CI, 2.1–10.7; $P = 0.0001$).

## Discussion

The prevalence of lactobacilli (18%) in our study was of the same magnitude as that reported by Grindefjord et al. (19), who found that 22% of children 2.5 years of age were colonized with lactobacilli. They also showed an association between early colonization of cariogenic microflora, mutans streptococci, and lactobacilli and an increased risk of developing dental caries in young children. Carlsson et al. (2) found lactobacilli only occasionally in children younger than 2 years, but after the age of 2 years the lactobacilli count increased, and the presence of these microbes was associated with caries in children aged 2–5 years.

Candida genera have been found to be the commonest yeast in the mouth (20). The proportion of carriers of candida in children varies from 29% to 70% (21, 20) and increases with age (3). Carriage of salivary yeasts and the carriage of both lactobacilli and yeasts predict significantly the 3-year caries increment (22). We found oral candida in 24% of the children whose mean age was 2.5 years, and we also noticed that pacifier-sucking increased the occurrence of the carriage of candida. Salivary lactobacilli were also increased among children using a pacifier. Both microbes are considered to be aciduric (4) and thus unfavorable to dental health.

The reason for the increased frequency of oral lactobacilli and candida counts in children using a pacifier may be the change in local environmental conditions in the mouth. A pacifier may affect oral sugar clearance in the same manner as removable dentures, which have been shown to contribute to less effective clearance (23). Reduction in clearance would prolong conditions of low pH in plaque and thereby favor the selection of aciduric microorganisms (24). In the study by Arendorf & Addy (8) a significant fall in salivary pH was found in the presence of a removable orthodontic appliance together with an associated increase in both the frequency and density of candidal colonization.

A pacifier may also increase the number of receptor sites available for microbial adhesion. It may directly affect plaque accumulation, increasing it in the same manner as when a partial denture is placed in a patient's mouth (25, 26). Such appliances could provide a suitable surface to which oral candida and lactobacilli could adhere and become established. It has also been suggested that a pacifier interferes with the mucous membrane, favoring candida colonization (11).

Prolonged bottle-feeding and breast-feeding have been shown to cause 'nursing caries' (27–29). In our survey the use of a nursing bottle at night had less influence on the colonization of both caries-associated microbes than a pacifier alone. Breast feeding did not affect the microbial counts.

The antibiotics used in the treatment of children's infections are usually given as syrup. The sweeteners are often fermentable, highly acidogenic sugars (30) and may therefore promote the colonization of aciduric microbes such as lactobacilli and candida. However, the influence of antibiotics on salivary microorganisms is probably transient.

Young children (<2 years) had more lactobacilli (25%) and candida (33%) in their saliva than children more than 2 years old (lactobacilli in 15% and candida in 19%). A borderline association between those microbes and pacifier and nursing bottle at night was found in the younger group (Tables 2 and 3). We therefore suggest that reasons for higher prevalences of salivary microbes in the younger group may be the common pacifier-sucking (73%) and the use of nursing bottle at night (54%).

In the young age group (children <2 years) respiratory infections seemed to promote the colonization of salivary microorganisms. The prevalence of the symptoms of these infections was also higher in these younger children (Table 1). Respiratory infections may predispose the patient to mouth-breathing, which leads to a dry mouth in the anterior area. A dry mouth is a good target for colonization of both salivary lactobacilli and yeasts (17).

The socioeconomic status of the family was not evaluated in this study. Although socioeconomic status has been shown to influence the caries risk in preschool children (31, 32), in a recent Finnish study the socioeconomic background of pacifier users did not differ from that of the total study population (15).

In Western industrialized countries most infants are pacifier- or thumb-suckers (33, 35). In the present study the prevalence of children who had sucked a pacifier (78%) was of the same magnitude as reported in Sweden by Larsson et al. (33) and by a recent Finnish study (34). The period of pacifier-sucking coincides with the development of the primary dentition and disturbs the occlusal development to a certain extent (13–15). To reduce the risk of cross-bite development in the primary dentition, it has been suggested that sucking habits should be brought under control by the age of 2 years (36). Some, however, recommend that the pacifier sucking habit should be broken by the time the child is 1 year old (35).

The results of the present study suggest that the use of a pacifier may lead to an increase of caries activity in the

early primary dentition, as indicated by increased occurrence of salivary caries-associated microorganisms. Therefore, we conclude that sucking a pacifier for a long time after the teeth have erupted may result in conditions in the oral cavity which may later turn out to be a risk to dental health.

## References


1. McCarthy C, Snyder ML, Parker RB. The indigenous oral flora of man. I. The newborn to the 1-year-old infant. Arch Oral Biol 1965;10:61–70.
2. Carlsson J, Grahnen H, Johnsson G. Lactobacilli and streptococci in the mouth of children. Caries Res 1975;9:333–9.
3. Somerville DA. The normal flora of the skin in different age groups. Br J Dermatol 1969;81:248–58.
4. Bratthall D, Carlsson P. Clinical microbiology of saliva. In: Tenovuo JO, editor. Human saliva: clinical chemistry and microbiology. Vol II. Boca Raton (FL): CRC Press Inc., 1989: 224–34.
5. Parvinen T. Stimulated salivary flow rate, pH and lactobacillus and yeasts concentrations in persons with different types of dentition. Scand J Dent Res 1984;92:412–8.
6. Marsh PD, Percival RS, Challacombe SJ. The influence of denture-wearing and age on the oral microflora. J Dent Res 1992;71:1374–81.
7. Addy M, Shaw WC, Hansford P, Hopkins M. The effect of orthodontic appliances on the distribution of candida and plaque in adolescents. Br J Orthod 1982;9:158–63.
8. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol 1985;12:360–8.
9. Lundström F, Krasse B. Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. Eur J Orthod 1987;9:109–16.
10. Manning DJ, Coughlin RP, Poskitt EME. Candida in mouth or on dummy. Arch Dis Child 1985;60:381–2.
11. Sio JO, Minwalla FK, George RH, Booth IW. Oral candida: is dummy carriage the culprit? Arch Dis Child 1987;62:406–8.
12. Larsson E. Dummy- and finger-sucking habits in 4-year-olds. Swed Dent J 1975;68:219–24.
13. Larsson E. The effect of dummy-sucking on the occlusion: a review. Eur J Orthod 1986;8:127–30.
14. Lindner A, Modéer T. Relation of sucking habits and dental characteristics in preschool children with unilateral cross-bite. Scan J Dent Res 1989;97:278–83.
15. Paunio O, Rautava P, Sillanpää M. The Finnish Family Competence Study: the effect of living conditions on sucking habits in 3-year-old Finnish children and the association between these habits and dental occlusion. Acta Odontol Scand 1993;51: 23–9.
16. Larmas M. Plaque-mediated disease. Basic and clinical studies on the value of salivary monitoring. Ann NY Acad Sci 1993;694: 252–64.
17. Parvinen T, Larmas M. The relation of stimulated salivary flow rate and pH to lactobacillus and yeast concentrations in saliva. J Dent Res 1981;12:1929–35.
18. Norusis MJ/SPSS Inc. SPSS Advanced Statistics™. Chicago (IL): SPSS Inc., 1992.
19. Grindefjord M, Dahllöf G, Ekström G, Höjer B, Modeer T. Caries prevalence in 2.5 year-old children. Caries Res 1993;27: 505–10.
20. Martin MV, Wilkinson GR. The oral yeast flora of 10-year-old schoolchildren. Saboraudia 1983;21:129–35.
21. Hodson JJ, Craig GT. The incidence of Candida albicans in the plaques of teeth of children. Dent Pract Dent Res 1972;22: 296–301.
22. Pienihäkkinen K, Scheinin A, Banoczy J. Screening of caries in children through salivary lactobacilli and yeasts. Scand J Dent Res 1987;95:397–404.
23. Hase JC, Birkhed D. Oral sugar clearance in elderly people with prosthodontic reconstructions. Scand J Dent Res 1991;99:333–9.
24. Bradshaw DJ, McKee AS, Marsh PD. Effect of carbohydrate pulses and pH on population shifts within oral microbial communities in vitro. J Dent Res 1989;68:1298–302.
25. Addy M, Bates JF. The effect of partial dentures and chlorhexidine gluconate gel on plaque accumulation in absence of oral hygiene. J Clin Periodontol 1977;4:41–7.
26. Addy M, Bates JF. Plaque accumulation following the wearing of different types of removable partial dentures. J Oral Rehabil 1979;6:111–7.
27. Ripa LW. Nursing caries: a comprehensive review. Pediatr Dent 1988;10:268–82.
28. Roberts GJ, Cleaton-Jones PE, Fatti LP, Richarson BD, Sinwel RE, Hargreaves JA, et al. Patterns of breast and bottle feeding and their association with dental caries in 1-to-4-year-old South African children. I. Dental caries prevalence and experience. Community Dent Health 1993;10:405–13.
29. Matee MIN, van't Hof MA, Maselle SY, Mikx FHM, van Palenstein Helderman WH. Nursing caries, linear hypoplasia, and nursing and weaning habits in Tanzanian infants. Community Dent Oral Epidemiol 1994;22:289–93.
30. Rekola M. In vivo acid production from medicines in syrup form. Caries Res 1989;23:412–6.
31. Gratrix D, Holloway PJ. Factors of deprivation associated with dental caries in young children. Community Dent Health 1994; 11:66–70.
32. Grytten J, Rossow I, Holst D, Steele L. Longitudial study of dental health behaviours and other caries predictors in early childhood. Community Dent Oral Epidemiol 1988;16:356–9.
33. Larsson E, Ögaard B, Lindsten R. Dummy-and finger-sucking habits in young Swedish and Norwegian children. Scand J Dent Res 1992;100:292–5.
34. Niemelä M, Uhari M, Hannuksela A. Pacifiers and dental structure as risk factors for otitis media. Int J Pediatr Otorhinolaryngol 1994;29:121–7.
35. Svedmyr B. Dummy sucking. A study of its prevalence, duration and malocclusion consequences. Swed Dent J 1979;3:205–10.
36. Modéer T, Odenrick L, Lindner A. Sucking habits and their relation to posterior cross-bite in 4-year-old children. Scand J Dent Res 1982;90:323–8.


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