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The distribution of periodontopathic bacteria among Japanese children and their parents

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Objective and background: It is not well known how periodontopathic bacteria colonize in the oral cavity during childhood. The purpose of this study was to investigate the distribution of periodontopathic bacteria in oral cavities of children and their parents and the relationship between the bacterial findings and clinical parameters.

Methods: Fifty-six children (mean age: 8.3 ± 3.5, range: 1–15 years), including 15 with deciduous dentition, 26 with mixed dentition and 15 with permanent dentition, and their parents participated in this study. Whole saliva and dental plaque of the children and whole saliva of their parents were collected for detection of seven species of periodontopathic bacteria (*Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis* (*Bacteroides forsythus*), *Campylobacter rectus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *Treponema denticola*) using the polymerase chain reaction method. Clinical parameters including simplified Oral Hygiene Index and Papillary-Marginal-Attachment Index were recorded for the children and their accompanied parents.

Results: The detection frequencies of *T. forsythensis*, *C. rectus*, *P. nigrescens*, *T. denticola*, *A. actinomycetemcomitans and P. gingivalis* in the oral cavities of children were 42.9%, 94.6%, 42.9%, 48.2%, 1.8% and 8.9%, respectively. *T. forsythensis*, *P. gingivalis* and *T. denticola* were detected more frequently in the saliva of parents (54.8%, 54.8%, 88.1%, respectively) than in the saliva of children (25.5%, 7.3%, 41.8%, respectively). Different detection frequencies of *P. nigrescens* were found among the oral cavities of children with deciduous, mixed and permanent dentitions. In mixed dentition, females harbored *T. forsythensis* more frequently than males did. Children who harbored *T. forsythensis*, *P. intermedia*, *P. nigrescens* and *T. denticola* showed high scores for oral debris measurement by simplified Oral Hygiene Index. *T. forsythensis*, *P. intermedia* and *P. nigrescens* were detected more frequently in children whose parents were positive for these pathogens than in children whose parents were negative.

Conclusions: High plaque retention seems to promote the colonization of periodontal pathogens in the oral cavities of children. *T. forsythensis*, *P. intermedia* and *P. nigrescens* were detected more frequently in the oral cavities of children whose parents already harbored these bacteria. Familial transmission of these bacteria is suggested.

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The initiation and progression of periodontitis are caused by accumulation of periodontopathic bacteria in the subgingival area. In particular, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis* (*Bacteroides forsythus*) (1) and *Porphyromonas gingivalis* have been suggested to play important roles in various forms of periodontal diseases (2–6). *Campylobacter rectus*, *Prevotella intermedia*/*Prevotella nigrescens* and *Treponema denticola* are also related to periodontal breakdown as the secondary group of periodontal pathogens (2, 3). How and when these microorganisms colonize in the oral cavity during childhood remains unclear. To study the distribution of periodontal pathogens among family members would be helpful to clarify the problem and to predict future initiation and progression of periodontal diseases (4, 5).

Polymerase chain reaction (PCR) is a highly sensitive and specific method to detect and identify bacteria in biological samples, and several bacterial findings by this method have been reported in periodontal research (7–13). A PCR-based diagnostic method can detect exceedingly low levels of oral bacteria and may be particularly valuable to determine the initial stages of oral colonization and distribution of pathogenic species.

A. actinomycetemcomitans and *P. gingivalis* are considered as exogenous pathogens, which are transmitted from subjects who harbor the pathogens to others (14). The vertical transmission of periodontal pathogens from parents to their children seems to be the most frequent pathway. Saliva is suspected to be the most possible vehicle in transmission of these periodontopathic bacteria (15). Once colonization of the periodontal pathogens occurs, these bacteria are continuously washed into saliva from the subgingival area. Thus whole saliva sample may offer a rapid and easy source for bacterial examination by employing sensitive detection techniques such as PCR (16). Asikainen *et al.* reported that samples from stimulated saliva and tongue were useful for identifying subgingival bacteria and they recommended sampling saliva as a non-invasive, inexpensive and easy method (17).

The purpose of this study was to examine the detection frequencies of periodontopathic bacteria in oral cavities of children in relation to their clinical parameters, and to compare the bacterial findings with those of their parents.

Material and methods

Subjects and clinical examination

Fifty-six children, aged 1–15 years old (8.3 ± 3.5 years old), including 15 children with deciduous dentition, 26 children with mixed dentition and 15 children with permanent dentition, and 79 parents (40.5 ± 5.1 years old) participated in the present study. All subjects had not taken antibiotics within the past 3 months. Informed consents were obtained from all the subjects and their parents for pursuing this experiment.

Clinical parameters including simplified Oral Hygiene Index (OHI-S) (18) and Papillary-Marginal-Attachment Index (PMA Index) (19, 20) of the children and the parents who accompanied them were recorded. Deepest probing depths of six representative teeth (tooth number 16, 21, 24, 36, 41, 44) were measured for 39 of 42 accompanied parents.

Bacterial sampling

Whole saliva and supragingival plaque samples were collected from all the children and whole saliva samples were collected from their parents. The parents who accompanied the children were instructed to collect the saliva of their spouses in a sterile plastic tube and bring it on the next appointment.

A detailed procedure for sample collection and processing has been described previously (16). Briefly, supragingival plaque was collected from each tooth using a sterile curette and then immersed in 0.5 ml distilled water. After vigorously mixing by vortex, the supragingival plaque sample was diluted 1:2 v/v in distilled water and washed twice with distilled water. Then the bacterial cell pellet was resuspended in 0.4 ml distilled water. Approximately 1 ml of expectorated whole saliva was collected from each individual in a sterile plastic tube. Then 0.5 ml of the whole saliva sample was diluted 1:2 v/v in distilled water and washed four times with distilled water. After the final wash, the bacterial cell pellet was reconstituted with 0.5 ml distilled water.

PCR detection

PCR detection was based on the amplification of signature sequences of the bacterial 16S rRNA genes. The primer sequences and PCR procedure were based on the report by Ashimoto *et al.* (9). The bacterial species examined were *A. actinomycetemcomitans*, *T. forsythensis*, *C. rectus*, *P. gingivalis*, *P. intermedia*, *P. nigrescens* and *T. denticola*. The DNA templates for PCR amplification were prepared by heating the bacterial samples at 100°C for 10 min followed by centrifugation to remove unbroken cells and large debris. The 50 μl PCR reaction mixture contained 5 μl of the sample, 5 μl of 10 × PCR buffer (Promega, Madison, WI, USA), 1.25 U *Taq* DNA polymerase (Promega), 0.2 mM of each deoxyribonucleotide (Pharmacia LKB, Piscataway, NJ, USA), 1.0 mM of each primer, and either 1.0 mM $MgCl_2$ for *A. actinomycetemcomitans*, *P. intermedia* and *P. nigrescens*, or 1.5 mM $MgCl_2$ for *T. forsythensis*, *C. rectus*, *P. gingivalis* and *T. denticola*. PCR amplification was performed in a DNA thermal cycler (PTC-200, MJ Research, Boston, MA, USA). The temperature profile for *A. actinomycetemcomitans*, *P. intermedia* and *P. nigrescens* included an initial step of 95°C for 2 min, followed by 36 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 2 min, and a final step of 72°C for 10 min. The PCR temperature profile for *T. forsythensis*, *P. gingivalis* and *T. denticola* included an initial step at 95°C for 2 min, followed by 36 cycles of 95°C for 30 s, 60°C for 1 min, 72°C for 1 min, and a final step of 72°C for 2 min.

PCR amplification products were analyzed by 1.0% agarose gel

electrophoresis. The gel was stained with 0.01 mg/l ethidium. A 100 bp DNA ladder digest (Promega) served as the molecular size marker.

Statistical analysis

Chi-squared test, unpaired *t*-test and ANOVA were employed for statistical analysis. The detection frequencies of periodontopathic bacteria in the oral cavities of children were compared with those of their parents by chi-squared test. The detection frequencies of periodontopathic bacteria in different dentitions and between different genders of children were also compared.

Unpaired *t*-test was used to compare the clinical parameters of children who were positive or negative for periodontopathic bacteria.

The clinical data of children with different dentitions were compared by ANOVA post-hoc test.

Results

The demographic data of the children and accompanied parents is shown in Table 1. The mean PMA Index was higher in permanent dentition stage than that in deciduous and mixed dentition stage ($p < 0.05$, $p < 0.01$, respectively). The mean score of the Simplified Debris Index (DI-S) of OHI-S was higher in the mixed dentition stage than in the deciduous dentition stage ($p < 0.05$). The mean score of the Simplified Calculus Index (CI-S) of OHI-S was higher in the mixed and permanent dentition stage than that in the deciduous dentition stage ($p < 0.01$). The average pocket depth of examined sites was 2.82 ± 0.94 for the accompanied parents, and only four accompanied parents had periodontal pockets deeper than 4 mm.

Bacterial detection frequencies in the oral cavities of children with different dentition stages are shown in Fig. 1. Periodontopathic bacteria, except *C. rectus*, were detected less frequently in deciduous dentition and more frequently in mixed dentition. There was a significant difference in the detection frequencies of *P. nigrescens* in the oral cavities of children with mixed, deciduous and permanent dentitions ($p < 0.05$). *C. rectus* was detected in all dentitions at a high and similar frequency.

Figure 2(a) shows the comparison of the Simplified Debris Index of OHI-S of children who either did or did not have periodontopathic bacteria in the oral cavities. Children who harbored *T. forsythensis*, *T. denticola*, *P. intermedia* and *P. nigrescens* showed significantly higher values for Oral Debris Scoring of OHI-S than children who did not harbor these bacteria ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.05$, respectively). These results implied that periodontopathic bacteria were detected more often in children with poor oral hygiene.

Scores for Oral Calculus Index of OHI-S and PMA Index of children with and without periodontopathic bacteria are shown in Figs 2(b) and (c). Children who harbored *P. nigrescens* exhibited significantly higher values for the Oral Calculus Index of the OHI-S and higher PMA Index than children who did not harbor the bacterium ($p < 0.01, p < 0.05$, respectively). The comparison between Debris Index and Calculus Index and periodontopathic bacteria yielded similar results, except for *A. actinomycetemcomitans*, which was detected even from children free from calculus and gingival inflammation.

The detection frequencies of periodontopathic bacteria in males and females with different dentitions indicated that females with mixed dentitions significantly harbored *T. forsythensis* more frequently than males with mixed dentitions did ($p < 0.01$) (Fig. 3).

Comparison of bacteria detected in the saliva samples of children and parents who accompanied them is shown in Fig. 4. The detection frequencies of *C. rectus* and *T. denticola* both from children and from parents were more than 40% (87.5%, 88.1% for *C. rectus*; 41.8%, 88.1% for *T. denticola*). *A. actinomycetemcomitans* was detected in 1.8% of the children and 7.1% of the parents who accompanied them. *P. gingivalis* was detected in only 7.1% of the children, although it was present in 54.8% of the parents who accompanied them.

The comparison of detection frequencies of periodontopathic bacteria in the oral cavities of children whose parents were positive or negative for bacteria is shown in Fig. 5. When the parents did not harbor *A. actinomycetemcomitans*, *T. forsythensis*, *P. gingivalis* and *P. intermedia,* these bacteria could not be detected in the children. On the other hand, *T. forsythensis*, *P. intermedia* and *P. nigrescens* were detected more frequently in the oral cavities of children whose parents harbored these pathogens ($p < 0.01$).

Table 1. The demographic data of chi1dren

	Deciduous dentition	Mixed dentition	Permanent dentition	(Accompanied parents)
Age (mean ± SD)	3.7 ± 1.9	8.6 ± 2.3	12.3 ± 2.6	39.5 ± 4.3
Number of males	8	9	8	2
Number of females	7	17	7	40
Mean PMA Index (mean ± SD)	0.73 ± 1.90	4.68 ± 4.59	9.00 ± 7.19[bc]	5.54 ± 6.59
Mean score of OHI, DI-S	0.64 ± 0.52	1.17 ± 0.48[a]	0.94 ± 0.58	10.17 ± 6.33
Mean score of OHI, CI-S	0.00 ± 0.00	0.06 ± 0.06[b]	0.06 ± 0.04[b]	5.81 ± 6.08

CI-S, Simplified Calculus Index; DI-S, Simplified Debris Index; OHI, Oral Hygiene Index; PMA Index, Papillary-Marginal-Attachment Index.
[a]Significantly different from deciduous dentition ($p < 0.05$, ANOVA post-hoc test).
[b]Significantly different from deciduous dentition ($p < 0.01$, ANOVA post-hoc test).
[c]Significantly different from mixed dentition ($p < 0.01$, ANOVA post-hoc test).

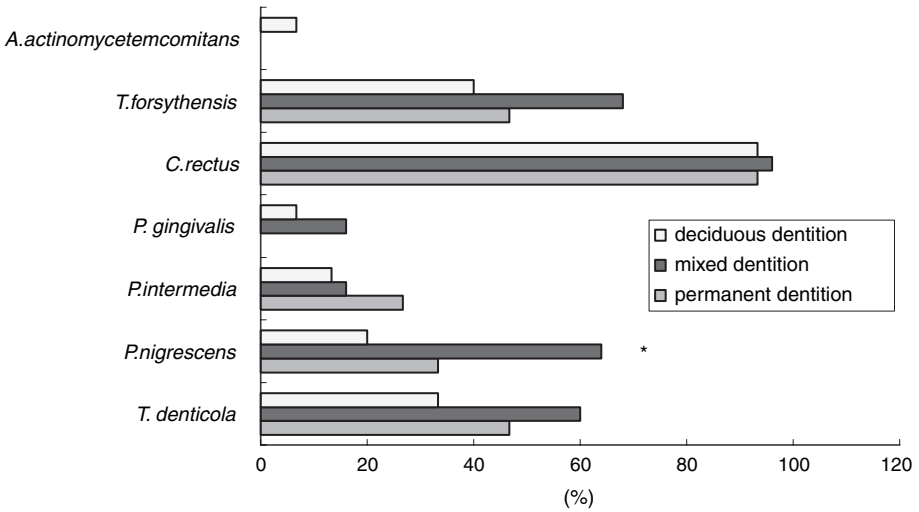

Fig. 1. Bacterial detection frequencies in the oral cavities of children with different dentitions (*$p < 0.05$).

Discussion

The usefulness of whole saliva for detection of periodontopathic bacteria in the oral cavity has been reported previously (16, 21). Saliva sampling is non-invasive, painless and easily accepted by patients. Subjects can collect their saliva by themselves using suitable sterilized plastic tubes (17). Therefore, PCR analysis of saliva samples was employed for detection of periodontopathic bacteria in the oral cavities of children and their parents in the present study.

It was, however, not certain whether the saliva of children contained a sufficient amount of periodontal pathogens for PCR detection, because the periodontal pockets of children are usually very shallow and it is not certain whether periodontal pathogens exist in their gingival sulcus. Furthermore, the bacterial flow-out from the crevice into saliva is low. Children with a large amount of dental plaque might possibly harbor periodontal pathogens because an anaerobic environment might be created within the accumulated plaque. The detection frequencies of bacteria in saliva and plaque in 51 children are four and three for *P. gingivalis*, 13 and 27 for *T. forsythensis*, 23 and 20 for *T. denticola*, 45 and 46 for *C. rectus*, seven and six for *P. intermedia*, and 12 and 20 for *P. nigrescens*. There were several cases that harbored bacteria in either saliva or plaque only. Therefore, plaque was also collected from the tooth surface to investigate the presence of periodontal pathogens in the oral cavities of children. In the case of their parents, enough amounts of bacteria for PCR detection are expected in saliva, and whole saliva is reported to be superior to pooled periodontal pocket samples to detect *P. gingivalis*, *P. intermedia*, *P. nigrescens* and *T. denticola* (16), and bacteria seemed to be transmitted via saliva to children. Therefore, only saliva samples were collected from parents.

Comparison of samples collected from children with deciduous, mixed and permanent dentitions indicated that the detection frequencies of *T. forsythensis*, *P. nigrescens* and *T. denticola* were comparatively higher in children with mixed dentition than in other children. Children with mixed dentitions also showed the highest score for Oral Debris of the OHI-S, which was significantly higher than those of children with deciduous or permanent dentitions ($p < 0.01$, unpaired *t*-test, Table 1). The bad oral hygiene at the changing phase from deciduous to permanent teeth might explain the higher detection frequencies of the periodontopathic bacteria. During the mixed dentition, increased gingival inflammation resulting from poor oral hygiene may cause formation of false pockets, which may contribute to the colonization of the periodontal pathogens and act as a bacterial reservoir. So professional oral care and examination in this transition period from deciduous to permanent teeth may be important for the prevention of further infection by periodontal bacteria.

Regarding the relationship between oral conditions and bacteria detected in the oral cavities of children, children who harbored *P. nigrescens* exhibited significantly higher PMA Index scores than children who did not harbor *P. nigrescens*. *P. nigrescens* may be associated with gingival inflammation in children. This is different from the study of Kamma *et al.*, who investigated the profile of subgingival microbiota in children with primary and mixed dentitions and reported that *P. intermedia* was found more frequently in bleeding sites (22, 23).

Children who harbored *T. forsythensis*, *T. denticola*, *P. intermedia* and *P. nigrescens* showed significantly higher values for Oral Debris of OHI-S than children who did not harbor these bacteria.

In mixed dentitions, females harbored *T. forsythensis* more frequently than males did. It is possible that the secretion of sex hormones might influence the growth of *T. forsythensis* in the oral cavities of females. The age of menstruation in Japanese girls has been reported to be at 10–11 years (24). In the present study, some girls with mixed dentition were in this age

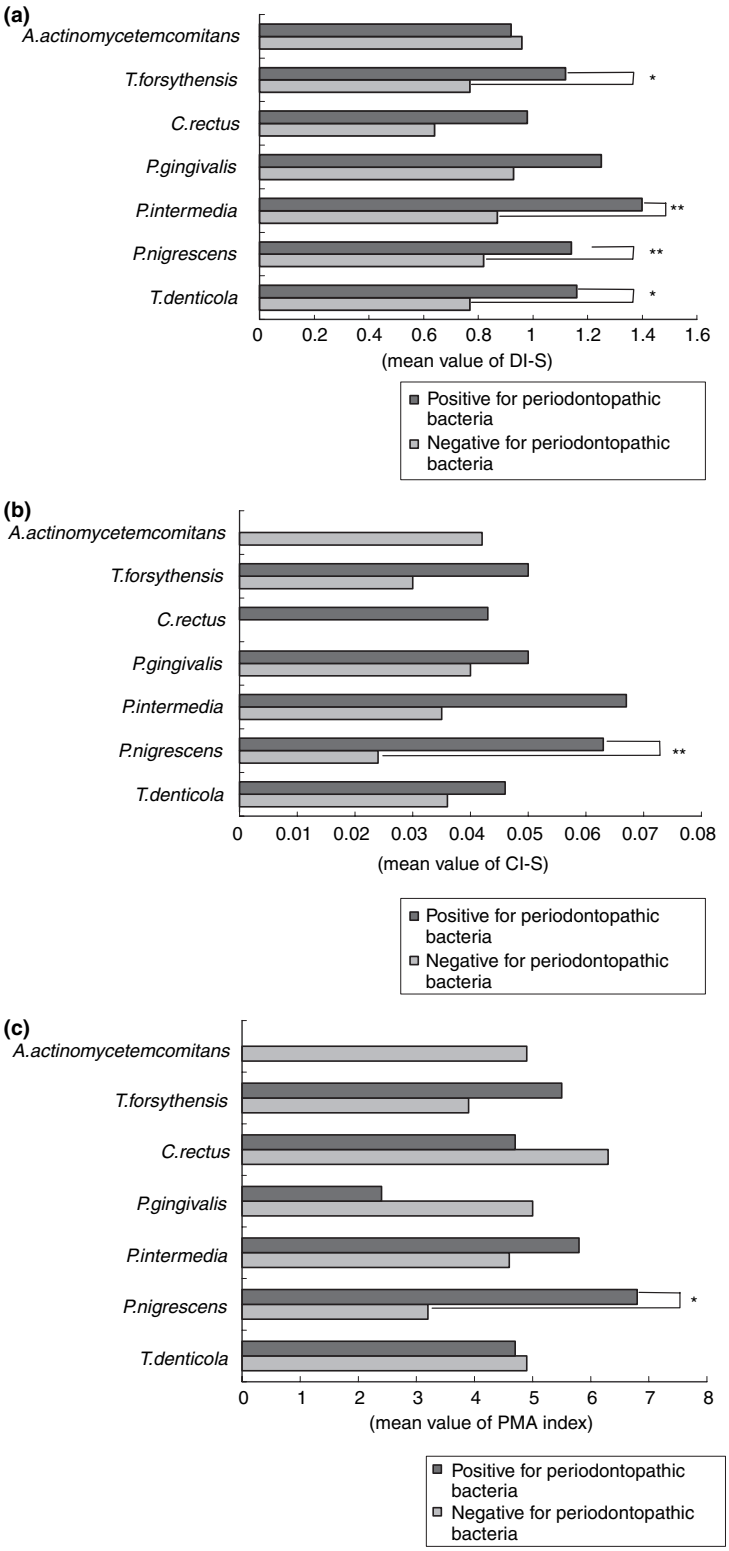

Fig. 2. (a) Simplified Debris Index (DI-S) of simplified Oral Hygiene Index (OHI-S) in the oral cavities of children with and without harboring of the periodontopathic bacteria (*$p < 0.05$, **$p < 0.01$). (b) Oral Calculus Index (CI-S) of OHI-S in the oral cavities of children with and without harboring of the periodontopathic bacteria (**$p < 0.01$). (c) Papillary-Marginal-Attachment Index (PMA Index) in the oral cavities of children with and without each harboring of periodontopathic bacteria (*$p < 0.05$).

group. Relationship between the colonization of periodontopathic bacteria and sex hormones has also been reported (25, 26).

Several studies have reported the possibility of periodontopathic bacterial transmission among family members (14, 27–36). The transmission of periodontal pathogens from parents to their children is reported to occur frequently (15, 31, 32). Similarity of the microbial detection pattern among family members was observed in our experiment (data not shown). Regarding the detection frequencies of periodontopathic bacteria in the saliva samples of children and parents who accompanied them, the children harbored these bacteria less frequently than their parents. The detection frequency of *P. gingivalis* in parents was over 50%, whereas that in children was less than 10%. Transmission of *P. gingivalis* was reported to be rather uncommon between spouses, despite their intimate cohabitation of several years (37). *P. gingivalis* may be difficult to transmit or may need a longer time to colonize. The detection frequencies of *C. rectus* and *T. denticola* were more than 40% both in the children and in their parents. *C. rectus* and *T. denticola* may be relatively easy to transmit and easier to colonize in the oral cavities of children.

The periodontopathic bacteria detected in the oral cavities of children were suspected to be transmitted from their parents through saliva or shared toothbrush (38, 39). Parents suffering from periodontitis may have a risk of transmitting periodontal pathogens to their children via saliva (40). In a study involving children aged 18–48 months, Yang *et al.* reported that when the mother had periodontitis, the probability that the children harbored periodontal pathogens was also significantly higher, especially in the case of *T. forsythensis* (41). We found in our experiment that children whose parents possessed *T. forsythensis*, *P. intermedia* and *P. nigrescens* harbored these bacteria significantly more frequently than children whose parents did not. Therefore, it is possible that these periodontopathic bacteria can be transmitted from parents to children

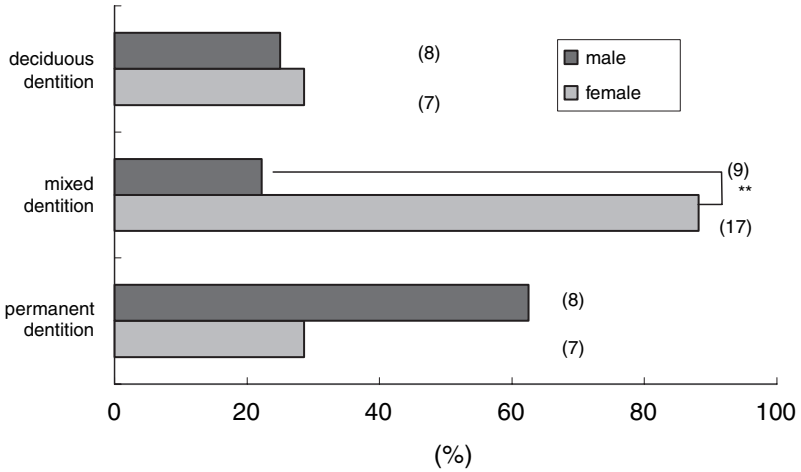

Fig. 3. Detection frequency of *Tannerella forsythensis* in males and females with different dentitions (**$p < 0.01$).

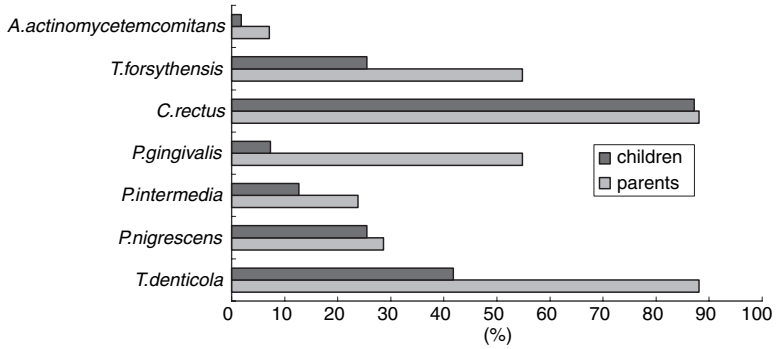

Fig. 4. Comparison of bacterial detection frequency in the saliva samples of children with parents who accompanied them.

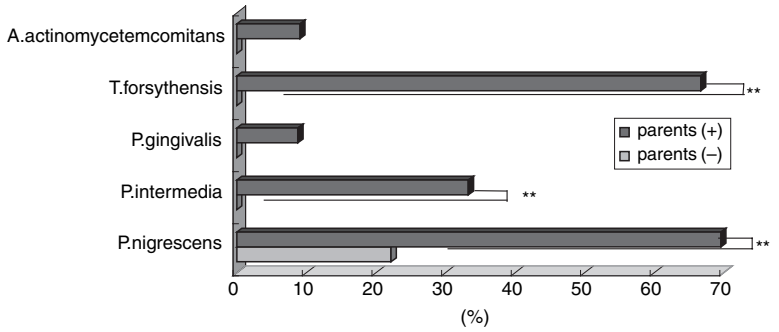

Fig. 5. Comparison of detection frequencies of periodontopathic bacteria in the oral cavities of children whose parents harbored the bacteria with those whose parents did not harbor them (**$p < 0.01$). There were no children whose parents did not harbor *Campylobacter rectus* or *Treponema denticola* in their oral cavities.

vertically. Our results indicate that some periodontopathic bacteria, such as *P. gingivalis*, are not so easily transmitted from parents to children. On the other hand, periodontopathic bacteria such as *T. denticola* and *C. rectus* seem to transmit among family members more often. However, in our study it was not investigated whether the parents and children harbored the same strains of periodontopathic bacteria. The transmission pathway may become more clear if we employ a sero- or genotyping method to distinguish the sero- or genotype of the same bacterial species (42–44). For genotyping, usually the isolation of target bacteria is necessary. In this case, isolation of periodontal pathogens in children seems to be difficult because only a small number of pathogens may be present. Employing of genotyping without culture is needed.

In order to prevent early onset of periodontitis in children, the parents should achieve and maintain periodontal health in addition to providing oral hygiene instructions to their children.

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