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ORIGINAL ARTICLE

Distribution of 10 periodontal bacterial species in children and adolescents over a 7-year period

K Nakano, E Miyamoto, K Tamura, H Nemoto, K Fujita, R Nomura, T Ooshima

Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan

OBJECTIVE: There is scant information available regarding the distribution of periodontal bacterial species in children and adolescents over an extended period. The purpose of this study was to compare bacterial profiles in the same individuals over a period of 7 years.

SUBJECT AND METHODS: Twenty-six children and adolescents from whom dental plaque and saliva specimens were obtained during both the first (1999–2000) and second (2006–2007) periods, were analyzed. Bacterial DNA was extracted from each specimen and the presence of 10 periodontal bacterial species was determined using a PCR method, with a focus on the red complex species of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*.

RESULTS: Subjects with red complex species in saliva specimens obtained during the second collection possessed a significantly higher number of total bacterial species than those without. The detection rate of the red complex species in the second collection period samples was significantly greater in subjects who had two or more species detected in samples taken during the first collection compared with the other subjects.

CONCLUSION: Subjects possessing red complex species may be at possible risk for infection with a high number of periodontal bacterial species during adolescent and younger adult years.

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Keywords: epidemiology; molecular biology; inflammatory diseases

Introduction

Gingivitis is commonly seen in children and in the early adolescence period, while the onset of periodontitis is generally identified in some subjects as early as late adolescence or during young adult years (Jenkins and Papapanou, 2001). Several periodontitis-related bacterial species have been reported, among which we recently focused on 10, including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Prevotella nigrescens*, *Campylobacter rectus*, *Eikenella corrodens*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, and *Treponema denticola*, based on results showing that their distribution in periodontitis patients was significantly different from that in healthy subjects (Alaluusua and Asikainen, 1988; Riviere *et al*, 1996; Darveau *et al*, 1997; Wara-aswapati *et al*, 1999; Lamell *et al*, 2000; Darby and Curtis, 2001).

The correlation of specific bacterial species with periodontitis has been investigated, with red complex species of *P. gingivalis*, *T. denticola*, and *T. forsythia* known to be major causative agents of periodontitis and highly associated with its severity (Socransky *et al*, 1998; Rôças *et al*, 2001). An analysis of specimens taken from Japanese school children aged 8–11 years showed that the presence of *P. gingivalis* or *T. forsythia* might be a possible risk marker for the onset of periodontal disease (Suda *et al*, 2003). In addition, *T. forsythia* and *C. rectus* may serve as risk markers for the onset of periodontitis, as demonstrated by the results of an analysis of specimens collected from 15-year-old Japanese subjects (Suda *et al*, 2004). Further, in a study of an adolescent population, *T. forsythia* was shown to be strongly associated with loss of attachment, which is considered to indicate an elevated risk of developing early chronic periodontitis (Hamlet *et al*, 2004).

The frequency of detection of these species, except for *C. rectus*, in periodontally healthy subjects in early childhood is considered to be extremely low, with the rates of *P. gingivalis* and *T. denticola* reported to be less than 1% (Ashimoto *et al*, 1996; Chen *et al*, 1997; Okada *et al*, 2000; Kimura *et al*, 2002; Okada *et al*, 2001; Ooshima *et al*, 2003; Tamura *et al*, 2005). On the other hand, *T. forsythia* has been reported to occur in a range of 8–33% of examined subjects (Ashimoto *et al*, 1996; Chen *et al*, 1997; Kimura *et al*, 2002; Okada *et al*, 2001;

Correspondence: Prof. T. Ooshima, Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel: +81 6 6879 2961, Fax: +81 6 6879 2965, E-mail: ooshima@dent.osaka-u.ac.jp
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Ooshima *et al*, 2003). In the present study, bacterial profiles in dental plaque and saliva specimens collected from subjects in 2006–2007 were retrospectively compared with those in specimens collected from the same subjects in 1999–2000, with a focus on the presence of the red complex group of species and total numbers of bacterial species.

Subjects and methods

Subjects and clinical specimens

All the study protocols were approved by the Ethics Committee of Osaka University Dental Hospital. In 1999 and 2000, we analyzed the occurrence of periodontal bacterial species in 192 healthy subjects (89 male and 103 female, 2–16 years old) who came to the Clinic of Pediatric Dentistry at Osaka University Dental Hospital. In the present study, we investigated only those ($n = 26$) who attended annual recall examinations until 2006–2007, by comparing specimens collected in 1999–2000 (first collection period) and in 2006–2007 (second collection period). Those subjects were confirmed to be without systemic diseases and had received no antibiotic medication for at least 3 months prior to each specimen collection. Figure 1 illustrates the timing of the first and second collection periods.

The mandibular left primary canine (LLC) and maxillary right second primary molar (URE) were selected as dental plaque specimen sites for subjects who had not experienced eruption of the permanent successors (group A, $n = 15$, six male, nine female), while the mandibular left canine (LL3) and maxillary right first molar (UR6) were chosen for those with eruption of both teeth (group B, $n = 11$, six male, five female). Dental plaque specimens were taken from the labial side of the selected teeth with sterile Gracey curettes and then suspended in sterile phosphate-buffered saline on ice. Following mouth-washing, expectorated whole saliva was also collected from each subject in a sterile plastic tube and immediately placed on ice. Both dental plaque and saliva specimens were transported to our laboratory immediately after collection for extraction of bacterial genomic DNA.

Clinical examinations

Clinical parameters, including probing depth, bleeding on probing (BOP), pus discharge, plaque index (Silness and Löe, 1964), and gingival index (Löe and Silness, 1963) were examined. Periodontal pocket depth (PD) was measured to the nearest millimeter at six points around the circumference of each tooth (mesio-, mid-, and disto-buccal; and disto-, mid-, and mesio-lingual) from the gingival margin to the deepest probing point, using a round-ended probe tip 0.4 mm in diameter. BOP was scored as (+) for immediate BOP or (−) for no bleeding, while pus discharge was scored as (+) for spontaneous pus discharge or (−) for no discharge.

Identification of periodontal bacterial species

Microbiological analyses were performed using methods described previously (Kimura *et al*, 2002; Ooshima *et al*, 2003). Briefly, subgingival plaque samples were collected

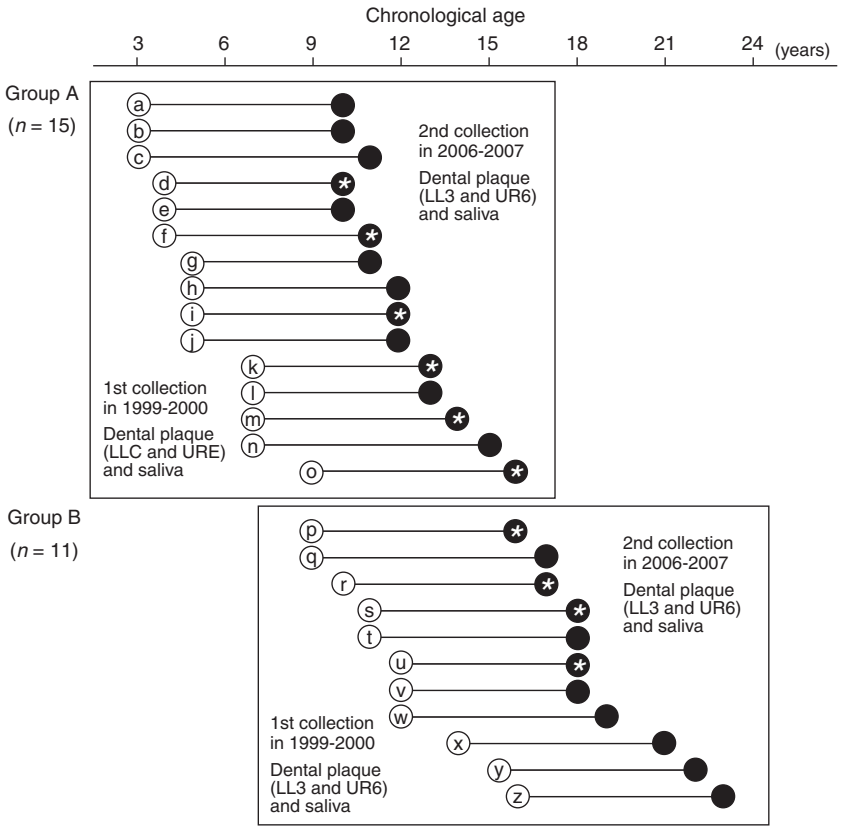

Figure 1 Illustration of sample collections and group divisions. Open and closed circles indicate the times when the first and second samples, respectively, were collected. Letters in open circles indicate individual subjects. LLC, mandibular left primary canine; URE, maxillary right second primary molar; LL3, mandibular left permanent canine; UR6, maxillary right permanent first molar. Asterisks indicate samples positive for red complex bacterial species

in sterile saline and centrifuged at 15 000 rpm (20 400 g) for 5 min to pellet the bacterial cells. Bacterial genomic DNA was extracted from each pellet using a DNA isolation kit (Puregene; Gentra Systems, Minneapolis, MN, USA). As for the saliva samples, expectorated whole saliva collected from each patient was mixed with Chelex 100 (Bio-Rad Laboratories, Hercules, CA, USA), then incubated at 56°C for 30 min, followed by boiling at 100°C for 10 min. Each sample was then centrifuged at 15000 rpm (20 400 g) for 20 min and the supernatants used as templates for PCR assays. The presence of 10 periodontitis-associated bacterial species, *P. gingivalis*, *T. denticola*, *C. ochracea*, *C. sputigena*, *P. intermedia*, *P. nigrescens*, *A. actinomycetemcomitans*, *T. forsythia*, *C. rectus*, and *E. corrodens*, was then analyzed in the specimens by PCR using species-specific sets of primers (Ashimoto *et al*, 1996; Conrads *et al*, 1996; Tran and Rudney, 1996; Watanabe and Frommel, 1996; Doungudomdacha *et al*, 2000).

Statistical analyses

Statistical analyses were performed using the computational software packages StatView 5.0 (SAS Institute Inc., Cary, NC, USA) and Prism 4 (GraphPad Software Inc., San Diego, CA, USA). The total numbers of bacterial species in dental plaque and saliva specimens from the same subjects were analyzed using a paired *t*-test. The correlation of total number of bacterial species in saliva specimens from the same subjects between the first and second collections was also analyzed using a paired *t*-test, while the total number of bacterial species and clinical parameters were evaluated by regression analysis. Similar to the analyses of red complex species, comparisons of total numbers of species in subjects with and without red complex species were performed using one-way ANOVA (Bonferroni multiple comparison test), with the odds ratio (OR) and 95% confidence interval (CI_{95}) calculated to determine any significant association between the total number of bacterial species in the first collection and presence of the red complex species in the second collection. In addition, Fisher's protected least-significant difference test was utilized to compare the detection frequency of each species between the group of subjects harboring red complex species and those without. $P < 0.05$ was considered to be significant.

Results

Detection of red complex species and total number of bacterial species in dental plaque and saliva specimens

Tables 1 and 2 show bacterial species detected in the dental plaque specimens from all subjects in the present study, while Table 3 presents those found in saliva specimens. *Tannerella forsythia* was the most frequently detected species among the three red complex species investigated in this study. There were three dental plaque specimens positive for *T. forsythia*, all from group B (subjects u and z in 1999, and subject r in 2006), while none of the saliva specimens obtained in 1999 were positive for *T. forsythia*, in contrast to nine obtained in 2006 that were shown to be positive (subjects d, f, k, m, o, p, r, s, and u) (Figure 1). The ages of subjects who provided positive specimens ranged from 10 to 18 years. Among all dental plaque and saliva specimens analyzed in this study, only two saliva specimens were found to be positive for *P. gingivalis* (subjects i and r in 2006) and none were positive for *T. denticola*.

As for *C. rectus*, its detection rate in saliva specimens was higher than that of the red complex species, with three and 13 saliva specimens taken in 1999 (subjects k, r, and z) and 2006 (subjects c, d, f, i, k, m, o, p, s, t, u, v, and z), respectively, shown to be positive. Eight of nine saliva specimens positive for *T. forsythia* were also positive for *C. rectus*. In addition, *E. corrodens* was found in four and nine saliva specimens collected in 1999 (subjects k, m, r, and t) and 2006 (subjects d, f, k, m, o, p, s, t, and v), respectively, of which seven specimens were also positive for both *T. forsythia* and *C. rectus*. Further, the dental plaque of subject r collected in 2006 was positive for *T. forsythia*, *C. rectus*, and *E. corrodens*.

The total numbers of bacterial species in both group A and group B ranged from 0 to 4, and no significant correlation was found between the first and second collections (data not shown). As for the saliva specimens, the total number in the first collection correlated with that in the second collection in group A ($P < 0.05$; paired *t*-test), whereas no significant correlation was found in group B (Figure 2). The total number of bacterial species identified in the saliva specimens of all subjects analyzed in this study was calculated to be 2.42 ± 0.28 (mean ± s.e.), which was significantly higher than that in the dental plaque specimens (1.46 ± 0.18, $P < 0.01$; paired *t*-test).

As for the correlation between bacterial species and clinical parameters, the total numbers of species was shown to be positively correlated with PD at the corresponding teeth ($P < 0.05$, $r = 0.23$). However, there were no other significant correlations between bacterial profiles and clinical parameters.

Correlation of total number of bacterial species and red complex bacterial species

The total number of bacterial species in each of the saliva specimens positive for red complex species was greater than four. Subjects with red complex species in saliva specimens from the second collection (RC+) were shown to possess a significantly greater number of total bacterial species than those without (RC−) ($P < 0.001$) (Figure 3). In addition, retrospective analyses of specimens from RC+ subjects collected in 1999–2000 showed that those subjects possessed a significantly greater number of total species than the RC− subjects ($P < 0.05$). *Prevotella nigrescens*, *C. rectus*, and *E. corrodens* were detected with significantly higher rates in RC+ subjects than those in RC− subjects ($P < 0.01$). When all samples obtained at the first collection were divided into two categories based on the number of total bacterial species, high (2 or more) and low (1 or 0), the detection rate of the red complex species at the second collection was significantly greater in the high group

Table 1 Clinical and microbiological analyses of dental plaque specimens from group A

	First collection							*Second collection*						
			Clinical parameters							*Clinical parameters*				
Patients	*Age*	*Location*	*PD*	*BOP/pus*	*PI*	*GI*	*Microbiological detection*	*Age*	*Location*	*PD*	*BOP/pus*	*PI*	*GI*	*Microbiological detection*
a	3	LLC	1	−/−	0	0	None	10	LL3	2	−/−	0	1	None
		URE	2	−/−	0	0	None		UR6	3	−/−	0	0	None
b	3	LLC	2	−/−	0	0	CS	10	LL3	3	−/−	0	0	None
		URE	2	−/−	0	0	CS/AA/CR/EC		UR6	4	−/−	0	0	AA
c	3	LLC	2	−/−	1	0	CO/CS/AA/EC	11	LL3	3	−/−	1	1	None
		URE	2	−/−	2	0	CO/CS/AA/EC		UR6	2	−/−	0	1	None
d	4	LLC	2	−/−	0	0	None	10	LL3	2	−/−	0	0	None
		URE	2	−/−	0	0	None		UR6	2	−/−	0	0	CS
e	4	LLC	2	−/−	1	1	None	10	LL3	1	−/−	1	0	None
		URE	3	−/−	1	1	CS		UR6	2	−/−	1	0	None
f	4	LLC	2	−/−	0	0	CO/CS/AA	11	LL3	1	−/−	1	0	CO/AA/CR
		URE	3	+/−	0	1	AA		UR6	3	−/−	1	1	PN/CR/EC
g	5	LLC	1	−/−	0	0	None	11	LL3	2	−/−	1	0	AA
		URE	2	−/−	0	0	None		UR6	4	−/−	0	0	AA
h	5	LLC	2	+/−	1	0	CS/PN/AA/CR	12	LL3	3	−/−	1	0	AA/CR
		URE	3	−/−	1	1	CS/EC		UR6	4	−/−	1	0	AA
i	5	LLC	2	−/−	0	0	PN/AA/CR/EC	12	LL3	4	−/−	1	0	PN/AA
		URE	3	−/−	1	1	CR		UR6	3	−/−	1	0	PN/AA/EC
j	5	LLC	3	−/−	1	1	CS/PN	12	LL3	2	−/−	0	0	None
		URE	3	−/−	1	1	None		UR6	2	−/−	1	0	None
k	7	LLC	3	+/−	1	1	CO/CS/AA	15	LL3	2	−/−	0	0	None
		URE	3	−/−	1	1	CO		UR6	2	−/−	0	0	None
l	7	LLC	2	−/−	0	0	None	13	LL3	3	−/−	1	0	CS/AA/CR/EC
		URE	2	−/−	0	0	EC		UR6	3	−/−	1	0	None
m	7	LLC	2	−/−	0	0	AA/CR/EC	14	LL3	3	−/−	0	1	None
		URE	3	−/−	0	0	CS/AA		UR6	3	−/−	0	1	None
n	7	LLC	2	+/−	1	0	CS	15	LL3	3	−/−	0	0	None
		URE	2	+/−	1	1	CS/AA/CR/EC		UR6	4	−/−	0	0	AA
o	9	LLC	2	−/−	1	0	CS	16	LL3	3	−/−	2	1	CR
		URE	2	−/−	1	0	CR/EC		UR6	3	−/−	2	1	CO/PN/CR

LLC, mandibular left primary canine; URE, maxillary right second primary molar; LL3, mandibular left permanent canine; UR6, maxillary right first permanent molar; PD, periodontal pocket depth; BOP, bleeding on probing; pus, pus discharge; PI, plaque index; GI, gingival index; CO, *Capnocytophaga ochracea*; CS, *Capnocytophaga sputigena*; PN, *Prevotella nigrescens*; AA, *Aggregatibacter actinomycetemcomitans*; CR, *Campylobacter rectus*; EC, *Eikenella corrodens*.

(OR 17.5, CI_{95} 1.2–250.4). Further, a significant increase in the total number of species in samples obtained in the second collection period was identified in the RC+ group when compared with the first collection period ($P < 0.05$).

Discussion

We previously reported the distribution frequency of the present 10 periodontal bacterial species in dental plaque and saliva specimens from 144 children not affected by periodontitis (Kimura *et al*, 2002; Ooshima *et al*, 2003). Data for a total of 192 subjects (aged 2–16) were collected in the period of 1999–2000 and included in the results of those studies. In the present study, dental plaque and saliva specimens were again collected from 26 of those 192 subjects in 2006–2007. To our knowledge, this is the first study that compared the detection of these 10 periodontal bacterial species in the same subjects over a long interval. It should be noted that all 26 subjects continue to visit our clinic two or three times a year, even though there are no signs or symptoms of periodontitis. A major reason for their good periodontal condition is considered to be due to their continuous participation in our recall system, in which not only periodical examinations are performed, but also professional tooth cleaning and brushing instructions are provided.

Analysis of the correlation between clinical parameters and presence of the bacterial species was anticipated to be difficult, as none of the subjects in this study were found to have periodontitis. However, there were several patients with PD of 4–5 mm who did not have attachment loss. Analysis of the subjects including the patients with gingivitis showed that PD and total number of bacterial species were positively correlated ($P < 0.05$). On the other hand, statistical analyses of the detection frequency of the 10 periodontal bacterial species in dental plaque and saliva specimens showed that five species, including *T. forsythia*, were frequently detected in saliva specimens, while only *P. nigrescens* had a significantly higher detection rate in the dental plaque specimens (Ooshima *et al*, 2003). In the present study, the total number of bacterial species in saliva specimens was significantly greater than that in dental plaque specimens ($P < 0.01$), which indicates that periodontal bacterial species can be more easily detected in saliva. In addition, the higher detection rate may have been

Table 2 Clinical and microbiological analyses of dental plaque specimens from group B

	First collection							*Second collection*						
			Clinical parameters							*Clinical parameters*				
Patients	*Age*	*Location*	*PD*	*BOP/pus*	*PI*	*GI*	*Microbiological detection*	*Age*	*Location*	*PD*	*BOP/pus*	*PI*	*GI*	*Microbiological detection*
p	9	LL3	3	−/−	1	0	CS/AA	16	LL3	3	−/−	0	0	None
		UR6	5	+/−	1	1	CO/CS/AA/EC		UR6	3	−/−	0	0	None
q	9	LL3	3	−/−	1	0	CS/PN	17	LL3	2	−/−	0	0	AA
		UR6	3	−/−	0	0	CS/PN		UR6	2	−/−	0	0	AA
r	10	LL3	2	−/−	0	1	AA	17	LL3	2	−/−	0	1	None
		UR6	3	−/−	2	0	CO		UR6	2	−/−	1	1	CO/CS/PI/PN/AA/TF/CR/EC
s	11	LL3	1	−/−	1	0	CO/CS/AA/EC	18	LL3	3	−/−	0	1	None
		UR6	3	−/−	1	0	CO/CS/AA/EC		UR6	3	−/−	0	1	None
t	11	LL3	2	−/−	0	0	CS	18	LL3	2	−/−	0	0	None
		UR6	3	−/−	0	0	CS/PN/AA		UR6	3	−/−	0	1	None
u	12	LL3	3	+/−	2	0	CO/CS/PN/AA/TF	18	LL3	3	−/−	0	0	CS/AA/EC
		UR6	3	+/−	1	1	CO/CS		UR6	3	−/−	0	0	CS/CR/EC
v	12	LL3	2	−/−	1	0	CO/CS/CR	18	LL3	2	−/−	1	0	AA/CR/EC
		UR6	3	−/−	2	1	None		UR6	3	−/−	1	1	PN/AA
w	12	LL3	3	−/−	2	1	None	19	LL3	2	−/−	1	0	None
		UR6	3	−/−	1	0	CO/PN/AA/CR		UR6	2	−/−	2	1	None
x	14	LL3	2	−/−	0	0	None	21	LL3	1	−/−	0	0	AA
		UR6	3	−/−	1	0	None		UR6	2	−/−	0	0	AA
y	15	LL3	2	−/−	1	0	CO/CS	22	LL3	2	−/−	0	0	AA
		UR6	3	−/−	1	1	CO/CS/PN/AA/EC		UR6	3	−/−	0	0	AA
z	16	LL3	3	−/−	1	0	CO	23	LL3	3	−/−	1	1	None
		UR6	3	−/−	1	0	CO/CS/PN/AA/TF		UR6	2	−/−	0	1	None

LL3, mandibular left permanent canine; UR6, maxillary right first permanent molar; PD, periodontal pocket depth; BOP, bleeding on probing; pus, pus discharge; PI, plaque index; GI, gingival index; CO, *Capnocytophaga ochracea*; CS, *Capnocytophaga sputigena*; PI, *Prevotella intermedia*; PN, *Prevotella nigrescens*; AA, *Aggregatibacter actinomycetemcomitans*; TF, *Tannerella forsythia*; CR, *Campylobacter rectus*; EC, *Eikenella corrodens*.

Table 3 Microbiological analyses of saliva specimens

	First collection		*Second collection*	
Patients	*Age*	*Microbiological detection*	*Age*	*Microbiological detection*
a	3	None	10	AA
b	3	None	10	None
c	3	CS	11	CR
d	4	CO/CS	10	CS/PI/PN/TF/CR/EC
e	4	CO/CS/AA	10	CO/CS/EC
f	4	AA	11	CO/CS/PN/AA/TF/CR/EC
g	5	None	11	CS/AA
h	5	CO/CS	12	CS/AA
i	5	CO/CS/AA	12	PG/CS/PN/AA/CR
j	5	None	12	None
k	7	CO/CS/PI/AA/CR/EC	15	CO/CS/PN/TF/CR/EC
l	7	None	13	CS
m	7	CO/CS/AA/EC	14	PN/TF/CR/EC
n	7	None	15	None
o	9	CO/CS/AA	16	CO/CS/PN/AA/TF/CR/EC
p	9	CS/AA	16	CO/CS/PN/TF/CR/EC
q	9	CO/AA	17	AA
r	10	CO/CS/PI/AA/CR/EC	17	PG/CS/AA/TF
s	11	CO/CS/AA	18	PN/TF/CR/EC
t	11	CO/CS/AA/EC	18	CR/EC
u	12	CO/CS/AA	18	CS/AA/TF/CR
v	12	CO/CS/PN/AA	18	CR/EC
w	12	None	19	None
x	14	CO/CS/AA	21	None
y	15	CO/CS	22	CS/AA
z	16	CO/CS/AA/CR	23	CR

PG, *Porphyromonas gingivalis*; CO, *Capnocytophaga ochracea*; CS, *Capnocytophaga sputigena;* PI, *Prevotella intermedia*; PN, *Prevotella nigrescens*; AA, *Aggregatibacter actinomycetemcomitans*; TF, *Tannerella forsythia*; CR, *Campylobacter rectus*; EC, *Eikenella corrodens*.

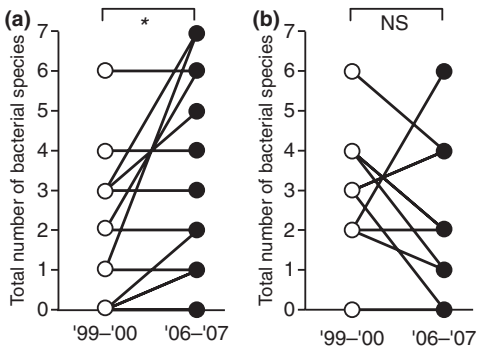

Figure 2 Change in total numbers of bacterial species in saliva specimens from the same subjects analyzed in 1999–2000 and 2006–2007. (**a**), group A; (**b**), group B. A paired *t*-test was performed (*$P < 0.05$; NS, not significant)

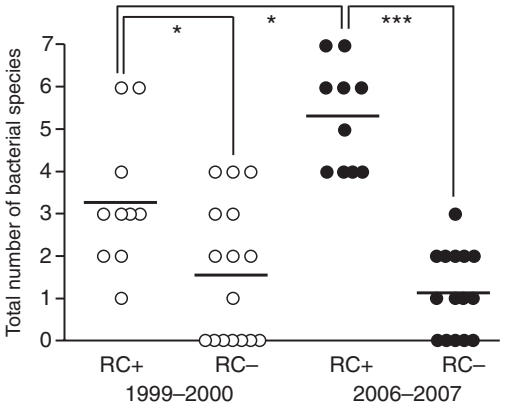

Figure 3 Total numbers of bacterial species in subjects with and without red complex bacterial species. Open and closed circles indicate samples collected in 1999–2000 and 2006–2007, respectively. Horizontal bars indicate the mean values for each group. A Bonferroni multiple comparison test was performed (*$P < 0.05$ and ***$P < 0.001$)

derived from the fact that saliva specimens are representative of the whole oral cavity.

The present retrospective analysis showed that the total number of bacterial species in saliva obtained in the first collection was positively correlated with that obtained in the second collection in group A ($P < 0.05$), whereas no significant correlation was found in group B (Figure 2). This finding implies that individuals who possess a relatively large number of periodontal bacterial species at an age younger than 10 years tend to have an even greater number of species several years later. The reason why the correlation was only found in our younger subjects (group A) might have been because of the low number of the specimens collected from the older subjects (group B).

As there were no subjects with periodontitis in this study, an analysis of the correlation between clinical parameters and presence of bacterial species was considered to be difficult. Therefore, we focused on the presence of red complex species, which has been reported to be correlated with gingival inflammation, based on studies of Japanese teenagers (Suda *et al*, 2003, 2004). It is of interest that our subjects with red complex bacterial species possessed a significantly greater number of bacterial species than those without ($P < 0.001$) (Figure 3). The main reason for this result may be because *P. nigrescens*, *C. rectus*, and *E. corrodens* were detected at significantly higher rates in the subjects with red complex species ($P < 0.01$). Further, a retrospective analysis of subjects who had the red complex species detected in the second collection specimens found significantly higher numbers of bacterial species in their first collection compared with the other subjects. Thus, individuals with a high number of total bacterial species may be at future risk for infection by red complex species.

Combinations of bacterial species simultaneously detected in the same specimens have been reported, with *T. forsythia* identified with *C. rectus* at a significantly high rate (Tamura *et al*, 2006). In addition, *C. rectus* was also frequently detected with *E. corrodens* in the same specimens. In our study, the total number of subjects positive for *T. forsythia* was 10, among whom eight were positive for *C. rectus*, and seven for both *C. rectus* and *E. corrodens*. These results indicate that the total number of bacterial species in individuals positive for *T. forsythia* tends to be high due to the simultaneous presence of other species.

In summary, our findings indicate that individuals under the age of 10 years who possess either at least one of the red complex species or *C. rectus* have an increased risk for possession of greater numbers of periodontal bacterial species during their adolescent or younger adult years.

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Author contributions

Kazuhiko Nakano and Takashi Ooshima planned the study. Kazuyo Fujita and Ryota Nomura collected the clinical specimens, and Eriko Miyamoto, Kiyoko Tamura and Hirotoshi Nemoto carried out molecular biological examinations followed by statistical analyses. Kazuhiko Nakano wrote the article under the supervision of Takashi Ooshima.

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