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The effect of fixed orthodontic appliances on the oral carriage of *Candida* species and Enterobacteriaceae

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SUMMARY The aim of this investigation was to evaluate the prevalence of *Candida* and Enterobacteriaceae in a group of adolescents during fixed orthodontic appliance (FOA) therapy. The experimental group was recruited from a larger sample of orthodontic patients who were clinically examined once to obtain baseline data before active treatment. The group comprised 27 subjects; 13 males, 14 females (mean age 15.5 ± 2.3 years). Thereafter, the experimental group was examined three times during a 3 month follow-up period after insertion of the FOA. The whole mouth plaque score was obtained, and the oral cavity was then sampled for *Candida* species and Enterobacteriaceae using three different microbiological culture techniques, namely the oral rinse, pooled plaque and the imprint culture.

A significant increase in candidal numbers was observed after FOA insertion when the imprint technique was used ($P < 0.001$), although the overall candidal prevalence rates obtained using the oral rinse and pooled plaque techniques did not demonstrate such a change. The predominant *Candida* species isolated was *C. albicans* and the number of coliform carriers significantly increased after the insertion of a FOA, as detected by the oral rinse ($P < 0.05$) and the pooled plaque ($P < 0.05$) techniques. In total, eight coliform species were isolated following FOA therapy compared with the three species isolated before insertion of the appliance. The results also revealed a significant increase in plaque index due to the introduction of a FOA. Taken together, these data imply that insertion of a FOA is likely to promote oral carriage of *Candida* and coliform species. Furthermore, it appears that routine oral hygiene instruction and information on appliance hygiene given to these patients may not necessarily reduce plaque accumulation and possible attendant effects. Further work with a larger cohort is required to confirm these findings.

Introduction

Many factors, both intrinsic and extrinsic, have an effect on the composition, metabolic activity, and pathogenicity of the highly diverse microflora of the mouth (Samaranayake, 2002). It has been reported that the presence of a fixed orthodontic appliance (FOA) greatly inhibits oral hygiene and creates new retentive areas for plaque and debris, which in turn predisposes to increased carriage of microbes and subsequent infection (Atack *et al.*, 1996). Thus, many have reported a link between an increased level of dental plaque in individuals treated with FOAs and the subsequent development of gingivitis (Truchot, 1991). Others have shown these subjects to be more prone to periodontal disease (Petti *et al.*, 1997) and loss of periodontal support (Hamp *et al.*, 1982). Several clinical studies also indicate an increasing incidence of incipient carious lesions on the facial and lingual aspects of the teeth (Øgaard, 1989) and increased Gram-positive bacterial counts in saliva (Rosenbloom and Tinanoff, 1991) during treatment with FOAs.

The high oral colonization by the fungal pathogen *Candida albicans* in individuals wearing either full or partial removable dentures is well documented (Budtz-Jörgensen, 1990). *Candida* species have also been isolated from dental plaque and caries (Hodson and Craig, 1972), human dental hard tissue (Sen *et al.*, 1997), and the subgingival flora (Rams and Slots, 1991). The co-existence of *Candida* species and Enterobacteriaceae (Gram-negative enteric bacteria colloquially known as coliforms) has been demonstrated in patients on cytotoxic therapy (Samaranayake *et al.*, 1984) and radiotherapy (Samaranayake *et al.*, 1988). Moreover, it is also known that enterobacteria, such as the *Klebsiella* species, promote candidal colonization of epithelia (Makrides and MacFarlane, 1982). Nevertheless, as yet, there have been no studies examining the colonization trends of *Candida* species or coliform bacteria in well-defined populations using FOAs. The aim of the present study was, therefore, to assess significant quantitative and qualitative alterations in the carrier rate of *Candida* and Enterobacteriaceae and the associated changes in the plaque index in a Chinese adolescent population with healthy mouths before and during FOA therapy.

Materials and methods

The experimental group

A cohort of 50 consecutive subjects was recruited to obtain baseline data before FOA therapy (visit 1). These patients (24 males and 26 females, mean age 15.5 ± 2.4

years) were analysed to evaluate gender differences, if any, in the oral carriage of *Candida*, Enterobacteriaceae and total bacterial counts (Table 1). After the evaluation of these parameters, the first 27 subjects (13 males and 14 females, mean age 15.5 ± 2.3 years) who were ready to receive orthodontic therapy were recruited for further studies, namely, oral yeast and coliform carriage on three sequential visits (visits 2–4), after insertion of the FOA.

The patients' dental stages were DS4 and M4 which implied that all the permanent teeth had erupted except the third molars (Björk *et al.*, 1964). None of the patients had a history of smoking, debilitating disease, antibiotic or steroid therapy (for a period of 6 months prior to the study), which affect oral candidal and coliform carriage (Samaranayake, 1990). Before the investigation, all individuals received oral hygiene and dental flossing instructions.

Clinical examination and plaque scores

All patients were examined by a single examiner (PK). The age, gender, dietary habits, dental and medical history (including drug therapy) and oral lesions were recorded. A complete oral examination of both soft and hard tissues was performed and clinical abnormalities, if any, were noted before the appliances were inserted. Plaque indices were recorded for each subject, as indicators of oral status, using the criteria of Silness and Löe (1964). The defined sites for plaque score in this study were the buccal and lingual aspects of the 12 anterior teeth and four first molars, yielding a total of 32 sites. This score was divided by 32 to derive a plaque score for each patient. Any subject with a high plaque index and poor oral hygiene was given thorough oral hygiene instructions and plaque control measures instituted prior to enrolment in the study.

Microbiological sampling and laboratory techniques

The oral candidal carriage rate of the subjects was evaluated using three different standardized techniques: the imprint culture, oral rinse, and the pooled plaque method.

Imprint culture. This technique, designed by Arendorf and Walker (1979), was used to determine the candidal colonization of the oral mucosa. In brief, sterile plastic foam pads (2.5 × 2.5 cm^2) were dipped in Sabouraud's broth and placed on the dorsum of the tongue for 60 seconds. The pad was then pressed firmly on to a Sabouraud's agar plate (Oxoid Ltd, Hampshire, UK) and incubated at 37°C for 48 hours. Candidal numbers were determined by visual counting and expressed as colony forming units per mm^2 (CFU/mm^2). Yeasts were identified by the Gram stain, germ tube test and the API 20C AUX assimilation tests (Bio-Mérieux, Marcyl' Etoile, France).

Oral rinse. This technique, described by Samaranayake *et al.* (1986), was used for the quantification of *Candida* species, Enterobacteriaceae, and total bacterial counts in the sample. In brief, the subjects were instructed to rinse their mouths with 10 ml of phosphate-buffered saline (0.1 M, pH 7.2) for 60 seconds. The rinse was then expectorated back into a universal container, on ice, and concentrated by centrifugation at 17 000g for 10 minutes. The supernatant was discarded, and the deposit resuspended in 1 ml of phosphate-buffered saline to obtain a concentrated oral rinse which was then inoculated on to Sabouraud's agar, MacConkey's agar, and blood agar (Oxoid Ltd) using the spiral plater system (Spiral Systems Marketing Ltd, Maryland, USA) to assess *Candida*, coliform and bacterial counts, respectively. For quantifying yeasts in a rinse sample, the Sabouraud's plates were incubated for 48 hours at 37°C, after which CFU were quantified using visual examination and the latter was multiplied by the dilution factor and the area to yield the CFU/ml of the original oral rinse sample. The yeasts obtained were then identified by methods described earlier (imprint culture method).

The oral Enterobacteriaceae and total bacterial counts in a rinse sample were ascertained by incubating MacConkey's agar and blood agar plates at 37°C for 24 hours and estimating the CFU/ml. The Enterobacteriaceae that grew in MacConkey's agar were examined using a Gram stain and by counting the CFU of the characteristic

Table 1 Candidal and Enterobacteriaceae prevalence (as a percentage), total bacterial count, and plaque index between males and females (n = 50, baseline data).

Sex	*Candida* species		Enterobacteriaceae		Mean total bacterial count CFU × 10^5/ml (SD)	Mean plaque index (SD)
	Oral rinse	Pooled plaque	Oral rinse	Pooled plaque		
Males (n = 24)	16.6	8.3	8.3	4.1	23.0 (21.8)	0.97 (0.47)
Females (n = 26)	26.9	23.0	7.7	0	15.1 (11.5)	0.81 (0.47)

No significant difference between males and females for all values quoted.
SD, standard deviation; CFU, colony forming units.

Gram-negative rods. The organisms were purified by subculture on blood agar and identified using the commercially available API 20E method, which is the standard identification system for Enterobacteriaceae and other Gram-negative rods (Samaranayake, 1999).

Pooled plaque. The pooled samples of supra- and subgingival plaque obtained from defined sites (see above) were mixed on a whirlimixer (Thermolyne Maxi Mix II, Iowa, USA) for 30 seconds; 25 µl were transferred to Sabouraud's agar and MacConkey's agar for yeast and Enterobacteriaceae isolation and quantification, respectively. The transferred inoculum was spread evenly on the plate and incubated at 37°C for up to 48 hours. *Candida* species and Enterobacteriaceae were then identified and the total cell counts in the plaque samples were enumerated as described above.

Statistical analysis

The median and percentiles of the total bacterial counts, plaque index, and *Candida* species and coliform numbers before and after insertion of FOAs were calculated and statistical differences evaluated by Wilcoxon's signed rank test, as the data were not normally distributed. Significant differences in the *Candida* species and coliform prevalence were analysed by McNemar's test.

Results

Baseline epidemiology of 50 patients

The mean prevalence of *Candida* species, Enterobacteriaceae, and the total bacterial counts of male and female patients before insertion of the FOA in the pilot study group are shown in Table 1. As the baseline data showed no significant gender differences in the oral carriage of *Candida*, coliform, and total bacteria, the first 27 subjects in the cohort were selected for the longitudinal study to collect samples on three sequential visits after insertion of the FOA. The baseline data of this experimental group did not significantly differ from the larger cohort of patients.

Total bacterial counts

A significant increase ($P \leq 0.01$) in the total bacterial counts (obtained by the oral rinse technique) was observed between the pre-insertion visit (visit 1) and the third post-insertion visit (visit 4). The median bacterial counts were 10.1×10^5 and 13.0×10^5 CFU/ml during the first and second post-insertion visits, respectively, and increased to 27×10^5 CFU/ml during the final visit, with an overall median increase of 14×10^5 CFU/ml after 3 months of FOA therapy. There was no significant increase in the total bacterial counts between the pre-insertion visit and the first and second post-insertion visits.

Plaque index

Analysis of the temporal differences in the plaque score for each individual showed a significant increase ($P < 0.05$) in the median plaque index between the pre-insertion visit (score = 1) and the second and third post-insertion visits (score = 1.25, for both visits). There was no significant difference in the median plaque index between the pre-insertion visit and the first post-insertion visit (score = 1 for both visits)

Quantitative analysis of candidal carriage

After FOA insertion, candidal carriage was examined using the oral rinse, imprint culture, and pooled plaque techniques. There was considerable individual variation in the candidal counts recovered from these samples before and after insertion of the FOA, irrespective of the sampling technique employed (Table 2). Analysis of these results indicated that candidal carriage did not significantly increase due to the insertion of the FOA, as detected by the oral rinse or pooled plaque techniques. However, a significant (27 per cent) increase in candidal carriage due to the insertion of the FOA was detected when the imprint culture technique was used ($P < 0.001$). In addition, five of the 27 individuals changed from a non-carrier of *Candida* species to a carrier after FOA insertion.

Qualitative analysis of candidal species

In the pre-insertion evaluation of the pooled data from all 50 subjects, the frequency of oral candidal carriage was 22 and 16 per cent, with the oral rinse and pooled plaque techniques, respectively. The predominant candidal species isolated using either technique was *C. albicans*,

Table 2 The number of subjects (percentage in brackets) positive for oral candidal carriage before (visit 1) and after (visits 2–4) the insertion of a fixed orthodontic appliance in 27 individuals, determined by the oral rinse, imprint culture, and pooled plaque techniques.

	Visit 1	Visit 2	Visit 3	Visit 4	Mean visits 2–4
Oral rinse	8 (29.6)	4 (14.8)	3 (11.1)	6 (22.2)	4.3 (16)
Imprint culture	2 (7.4)	8 (29.6)	7 (25.9)	7 (25.9)	7.3 (27)
Pooled plaque	6 (22.2)	5 (18.5)	6 (22.2)	6 (22.2)	5.6 (21)

while *C. guilliermondii* and *C. tropicalis* were also isolated from two different individuals.

Similarly, the predominant candidal species isolated post-insertion was also *C. albicans*; recovered on 83–87 per cent of occasions, during all three patient visits after insertion of the appliance (Table 3). Other *Candida* species found during these sampling sessions were *C. parapsilosis*, *C. guilliermondii* and *C. tropicalis*; each recovered on a single occasion.

Quantitative analysis of coliform carriage

The number of subjects with coliform carriage before (visit 1) and after FOA insertion (visits 2–4), determined by both the oral rinse and the pooled plaque techniques, are shown in Table 4. There was considerable individual variation in bacterial isolation among individuals. There was no significant difference between the quantitative yield of Enterobacteriaceae (detected either by the oral rinse or the pooled plaque technique) recorded before and after insertion of the FOA (data not shown). Nevertheless, statistical analysis of the prevalence rates revealed that there was a significant increase ($P \leq 0.05$) in the number of individuals with coliform carriage after insertion of the FOA irrespective of the technique used.

Qualitative analysis of coliform carriage

The pre-insertion coliform carriage was 6 and 2 per cent with the oral rinse and pooled plaque techniques, respectively. Three species of Enterobacteriaceae, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterobacter sakazakii* were isolated by the oral rinse technique, while only *Klebsiella pneumoniae* was detected by the pooled plaque method.

The mean frequency of isolation and the identity of coliform species from the experimental subjects during the three post-insertion visits are shown in Table 5. The predominant coliform species isolated from the group using either the oral rinse or the pooled plaque technique were *Klebsiella pneumoniae* (recovered on 36.3 per cent of occasions; 8/22) and *Enterobacter sakazaki* (isolated on 30.8 per cent of occasions; 4/13), respectively. The other coliform species isolated by both methods were *Enterobacter cloacae*, *Enterobacter gergoviae*, *Pseudomonoas aeruginosa*, *Enterobacter agglomerans*, *Acinetobacter* and *Yersinia.* The isolation frequency of Enterobacteriaceae was higher with the oral rinse technique than with the pooled plaque technique.

Table 3 The identity and frequency [number of occasions isolated* (percentage)] of *Candida* species isolated from the experimental subjects (27 individuals) after insertion (three visits) of fixed orthodontic appliances.

	Oral rinse	Pooled plaque	Imprint culture
C. albicans	18 (85.7)	20 (86.9)	20 (83.3)
C. parapsilosis	1 (4.7)	1 (4.3)	4 (16.6)
C. guilliermondii	1 (4.7)	0 (0)	0 (0)
C. tropicalis	1 (4.7)	2 (8.7)	0 (0)

*Some individuals carried more than a single *Candida* species.

Discussion

This study, which investigated microflora during fixed orthodontic therapy, indicates that the wearing of such appliances leads to increased carriage and considerable changes in the oral bacterial population, possibly due to the appliance-induced ecological alterations within the oral cavity. An initial low plaque index and total bacterial count for the baseline patient group was not surprising, as the participants were required to establish good oral hygiene prior to the experiment. However, after insertion of a FOA, a 10 per cent mean increase in the plaque index in the test group was noted. A significant increase in the plaque index was observed between the first and third, and the first and fourth visits.

The presence of orthodontic attachments on the labial and lingual surfaces of these teeth is likely to be the reason for this observation, as they interfere with thorough brushing of the gingival area. Similar changes in plaque accumulation during orthodontic treatment with removable (Addy *et al.*, 1982; Arendorf and Addy, 1985) and fixed appliances have been reported by several authors (Boyd, 1983; Alexander, 1991). Furthermore, the presence of rough-surfaced bonding material acting as a plaque trap and a gingival irritant (Scheie *et al.*, 1984) may have played a contributory role.

Table 4 The mean prevalence rate [*n* (percentage)] of Enterobacteriaceae in the experimental group (27 individuals) before (visit 1) and after (visits 2–4) the insertion of a fixed orthodontic appliance, determined by the oral rinse and pooled plaque techniques.

	Visit 1	Visit 2	Visit 3	Visit 4	Mean visits 2–4
Oral rinse	3 (11.1)	7 (25.9)	6 (22.2)	6 (22.2)	6.3 (23.4)
Pooled plaque	1 (3.7)	4 (14.8)	5 (18.5)	3 (11.1)	4.0 (14.8)

Table 5 The identity and frequency of isolation [number of occasions isolated (percentage)] of Enterobacteriaceae from the experimental group (27 individuals) after insertion (three visits) of fixed orthodontic appliances.

	Oral rinse	Pooled plaque
Klebsiella pneumoniae	8 (36.3)	2 (15.4)
Enterobacter sakazakii	3 (13.6)	4 (30.8)
Enterobacter cloacae	4 (18.1)	1 (7.7)
Enterobacter gergoviae	2 (9.0)	2 (15.4)
Pseudomonas aeruginosa	2 (9.0)	1 (7.7)
Enterobacter agglomerans	1 (4.5)	1 (7.7)
Acinetobacter species	1 (4.5)	1 (7.7)
Yersinia species	1 (4.5)	1 (7.7)
Total isolated	22	13

In contrast to these observations, Sinclair *et al.* (1987) showed no significant difference in plaque accumulation between pre-treatment and the insertion of FOAs in 13 adolescents. This finding has been supported by Davies *et al.* (1991) who evaluated the occlusal status, dental health, and sociopsychological development of a cohort of 1015 adolescents accepted for orthodontic treatment. The latter group demonstrated a significant reduction in all plaque and gingival indices between baseline and the 3 year examination in both the orthodontically treated and untreated groups. They concluded that behavioural factors rather than the orthodontic treatment itself were responsible for the additional gain in oral hygiene and gingival health experienced by the patients fitted with orthodontic appliances. Similar improvements in oral hygiene and gingival health in adolescents of this age range have been reported previously (Sutcliffe, 1972). However, a relatively recent survey performed by Kwan (1992) in 760 adolescents in Hong Kong from lower income groups, demonstrated a significant increase in the plaque index after insertion of a FOA, confirming the earlier hypothesis that in addition to the patient's attitude and behaviour, social class may also be a contributory factor in controlling plaque development. Hence, the significant increase in the plaque index after insertion of a FOA in the present study could partly be due to the patient's attitude and behaviour, as well as the presence of a FOA which made it difficult to keep the teeth clean. Thus, although an orthodontic appliance may have a detrimental effect upon plaque control, this can be minimized by regular advice and instruction, which may have a lasting effect.

Possibly as a consequence of the increased plaque index, a concomitant high median bacterial count of 27×10^5 CFU/ml was noted in the experimental group after 3 months of FOA therapy (visit 4). Although different sampling techniques were performed, the increase in the total bacterial count observed in the present study tends to confirm the results of previous investigators (Rosenbloom and Tinanoff, 1991; Sukontapatipark *et al.*, 2001). It implies that treatment with a FOA may alter the ecological environment in the oral cavity by introducing new stagnant areas available for bacterial colonization and retention of substrates. The appliances may also interfere with oral hygiene practice and cover considerable parts of the tooth surfaces with metal and composite materials, thereby increasing plaque retention.

A significant increase in candidal density after FOA insertion was also found when the imprint technique was used, although the overall candidal prevalence rates obtained using the oral rinse and pooled plaque techniques did not demonstrate such a change. This is not surprising, as it is accepted that the oral rinse technique is the most sensitive for evaluating oral yeast and coliform carriage and the imprint technique for the localization of yeast growth (Samaranayake *et al.*, 1986). On the other hand, Arendorf and Walker (1979) have shown that the presence of a prosthesis or an appliance increases candidal numbers, not only at the occluded site, but at all mucosal sites sampled. Arendorf and Addy (1985) investigated 33 patients who underwent removable orthodontic appliance therapy and found a direct relationship between the presence of a removable orthodontic appliance and *Candida* species.

In the present study, there were changes from a non-carrier of *Candida* species to a carrier state after FOA insertion in some individuals. Thus, it may be postulated that foreign objects, including dental prostheses or appliances, alter the oral environment by mechanisms as yet unknown, such that the proliferation of organisms, such as *Candida* species, is encouraged. There is, however, no convincing evidence that FOA insertion will change a non-carrier state into a carrier state. Longer-term investigations are necessary to test this hypothesis.

Of the *Candida* species isolated, the most predominant was *C. albicans*, while *C. parapsilosis*, *C. tropicalis*, and *C. guilliermondii* were less frequent. This supports the finding that *C. albicans* is the single most predominant candidal species in the oral cavity (Wright *et al.*, 1985). The data also confirm previous findings that more variant *Candida* species can be isolated using the oral rinse technique than the imprint culture or pooled plaque technique (Samaranayake *et al.*, 1986).

From the literature it does not appear that a qualitative evaluation of Enterobacteriaceae in patients with FOAs has been documented previously. Enterobacteriaceae are a group of Gram-negative bacteria usually found in small numbers among the oral commensal flora, and wide regional and ethnic variations in oral coliform carriage have been reported (Sedgley and Samaranayake, 1994a). The study by Philpot *et al.* (1980) from the Asian region shows a coliform carriage prevalence rate of 4 per cent for Malaysian children. This agrees with data from Hable *et al.* (1971), who studied 490 healthy Minnesota children under 16 years of age and found a 4.7 per cent prevalence rate for Enterobacteriaceae. In

an investigation of coliform prevalence in the plaque of 105 subjects in Scotland among young Caucasian students, the isolation rate was 5 per cent (Leitch *et al.*, 1991). The coliform prevalence rate in the present study was higher and this may be explained by the variations in geographical location, ethnicity, and age.

The prevalence rate of Enterobacteriaceae may change according to the ecology of the mouth, such as denture wearing. Marsh *et al.* (1992) found Enterobacteriaceae in only three of 120 (2.5 per cent) partial denture wearing Caucasian subjects aged 60–80 years of age or above. On the contrary, a higher (32 per cent) coliform prevalence rate in 300 community-dwelling ethnic Chinese (mean age 56.3 years) has been reported from a previous study in Hong Kong. Of the Enterobacteriaceae isolated in the latter investigation *Enterobacter cloacae* and *Klebsiella pneumoniae pneumoniae* were the most common species (Sedgley and Samaranayake, 1994b). The results of the current study indicate a lower coliform prevalence rate of 11 and 23 per cent, before and after FOA insertion, using the oral rinse sampling technique. These results are nevertheless comparable with the 14 per cent oral coliform prevalence rate in 91 8-year-old Hong Kong children reported by Sedgley *et al.* (1997). However, the prevalence rate was high in the current study compared with the results of Marsh *et al.* (1992), and this may be due to the disparity in age difference and ethnicity of the two groups; the present group consisted of 12–20-year-old Chinese adolescents, while in the study by Marsh *et al.* (1992) the subjects were an older group of Caucasians (60–80 years).

In total, eight coliform species, including *Klebsiella pneumoniae*, *Enterobacter sakazakii*, *Enterobacter cloacae*, *Enterobacter gergoviae*, *Pseudomonas aeruginosa*, *Enterobacter agglomerans*, *Acinetobacter*, and *Yersinia* species, were isolated following FOA therapy in comparison with the three species (*Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterobacter sakazakii*) isolated before insertion of the appliance. Hence, it is possible that a FOA may affect oral coliform colonization and carriage in adolescents. Interestingly, in a recent study where the bacterial composition of dental plaque deposits on two orthodontic bracket types (metallic and ceramic) were compared using 'Checkerboard' DNA–DNA hybridization analysis, 37 bacterial species were isolated, implying that the appliance surface may modify the oral environment and help colonize non-indigenous bacteria (Anhoury *et al.*, 2002). However, further longitudinal investigations with carefully designed methods are needed to clarify this hypothesis.

Conclusions

Treatment with a FOA may alter the ecology in the oral cavity by introducing new stagnant areas available for colonization and retention of Enterobacteriaceae. The results confirm this by indicating that FOAs have a direct effect upon the plaque index and the total bacterial count and an adverse and transient effect upon the prevalence and density of candidal and coliform carriage in this group of adolescents. The appliances may also interfere with oral hygiene practice as they cover considerable parts of the tooth surfaces with metal and composite materials. In clinical terms, these findings indicate that regular advice and routine instruction in oral and appliance hygiene given to this group of patients did not overcome completely the possible detrimental effects of plaque accumulation. Hence, particular attention has to be paid to the plaque control of patients undergoing FOA therapy, if potential adverse effects are to be prevented.

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References

Addy M, Shaw W C, Hansford P, Hopkins M 1982 The effect of orthodontic appliances on the distribution of *Candida* and plaque in adolescents. British Journal of Orthodontics 9: 158–163

Alexander S A 1991 Effects of orthodontic attachments on the gingival health of permanent second molars. American Journal of Orthodontics and Dentofacial Orthopedics 100: 337–340

Anhoury P, Nathanson D, Hughes C V, Socransky S, Feres M, Chou L L 2002 Microbial profile on metallic and ceramic bracket materials. Angle Orthodontist 72: 338–343

Arendorf T, Addy M 1985 Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. Journal of Clinical Periodontology 12: 360–368

Arendorf T M, Walker D M 1979 Oral candidal populations in health and disease. British Dental Journal 147: 267–272

Atack N E, Sandy J R, Addy M 1996 Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. Journal of Periodontology 67: 78–85

Björk A, Krebs A, Solow B 1964 A method for epidemiological registration of malocclusion. Acta Odontologica Scandinavica 22: 27–41

Boyd R L 1983 Longitudinal evaluation of a system for self-monitoring plaque control effectiveness in orthodontic patients. Journal of Clinical Periodontology 10: 380–388

Budtz-Jörgensen E 1990 *Candida*-associated denture stomatitis and angular cheilitis. In: Samaranayake L P, MacFarlane T W (eds) Oral candidosis. Wright, London, pp. 156–183

Davies T M, Shaw W C, Worthington H V, Addy M, Dummer P, Kingdon A 1991 The effect of orthodontic treatment on plaque and gingivitis. American Journal of Orthodontics and Dentofacial Orthopedics 99: 155–161

Hable K A, Washington II J A, Herman Jr E C 1971 Bacterial and viral throat flora. Comparison of findings in children with acute upper respiratory tract disease and in healthy controls in winter. Clinical Pediatrics 10: 199–203

Hamp S E, Lundström F, Nyman S 1982 Periodontal conditions in adolescents subjected to multiband orthodontic treatment with controlled oral hygiene. European Journal of Orthodontics 4: 77–86

Hodson J J, Craig G T 1972 The incidence of *Candida albicans* in the plaques of teeth of children. Dental Practitioner and Dental Record 22: 296–301

Kwan E 1992 Oral health status of 13 and 15 year-old secondary school children in Hong Kong. Thesis, University of Hong Kong

Leitich J A, Lang M, MacFarlane T W, MacKenzie D 1991 The prevalence and distribution of yeasts and coliform in dental plaque. Journal of Dental Research 70: 709 (abstract)

Makrides H C, MacFarlane T W 1982 Effect of commensal bacteria on the adherence of *Candida albicans* to epithelial cells *in vitro*. Microbios Letters 21: 55–60

Marsh P D, Percival R S, Challacombe S J 1992 The influence of denture-wearing and age on the oral microflora. Journal of Dental Research 71: 1374–1381

Øgaard B 1989 Prevalence of white spot lesions in 19-year-olds. A study on untreated and orthodontically treated persons five years after treatment. American Journal of Orthodontics and Dentofacial Orthopedics 96: 423–427

Petti S, Barbato E, Simonetti D'Arca A 1997 Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. Microbiologica 20: 55–62

Philpot C R, McDonald P J, Chai K H 1980 Significance of enteric Gram-negative bacilli in the throat. Journal of Hygiene (London) 85: 205–210

Rams T E, Slots J 1991 *Candida* biotypes in human adult periodontitis. Oral Microbiology and Immunology 6: 191–192

Rosenbloom R G, Tinanoff N 1991 Salivary *Streptococcus mutans* levels in patients before, during, and after orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics 100: 35–37

Samaranayake L P 1990 Host factors and oral candidosis. In: Samaranayake L P, MacFarlane T W (eds) Oral candidosis. Wright, London, pp. 115–119

Samaranayake L P (ed.) 1999 Enterobacteria. In: Essential microbiology for dentistry. Churchill Livingstone, London

Samaranayake L P (ed.) 2002 Essential microbiology for dentistry, 2nd edn. Churchill Livingstone, Edinburgh

Samaranayake L P *et al.* 1984 The oral carriage of yeasts and coliforms in patients on cytotoxic therapy. Journal of Oral Pathology and Medicine 13: 390–393

Samaranayake L P, MacFarlane T W, Lamey P J, Ferguson M M 1986 A comparison of oral rinse and imprint sampling techniques for the detection of yeast, coliform and *Staphylococcus aureus* carriage in the oral cavity. Journal of Oral Pathology and Medicine 15: 386–388

Samaranayake L P *et al.* 1988 The effect of chlorhexidine gluconate and benzydamine mouthwashes on mucositis induced by therapeutic irradiation. Clinical Radiology 39: 291–294

Scheie A A, Arneberg P, Krogstad O 1984 Effect of orthodontic treatment on prevalence of *Streptococcus mutans* in plaque and saliva. Scandinavian Journal of Dental Research 92: 211–217

Sedgley C M, Samaranayake L P 1994a Oral and oropharyngeal prevalence of Enterobacteriaceae in humans: a review. Journal of Oral Pathology and Medicine 23: 104–113

Sedgley C M, Samaranayake L P 1994b The oral prevalence of aerobic and facultatively anaerobic gram-negative rods and yeasts in Hong Kong Chinese. Archives of Oral Biology 39: 459–466

Sedgley C M, Samaranayake L P, Chan J C Y, Wei S H Y 1997 A 4 year longitudinal study of the oral prevalence of enteric gram-negative rods and yeasts in Chinese children. Oral Microbiology and Immunology 12: 183–188

Sen B H, Safavi K E, Spångberg L S 1997 Colonization of *Candida albicans* on cleaned human dental hard tissues. Archives of Oral Biology 42: 513–520

Silness J, Löe H 1964 Periodontal disease in pregnancy II: correlation between oral hygiene and periodontal condition. Acta Odontologica Scandinavica 22: 121–135

Sinclair P M, Berry C W, Bennett C L, Israelson H 1987 Changes in gingiva and gingival flora with bonding and banding. Angle Orthodontist 57: 271–278

Sukontapatipark W, El-Agroudi M A, Selliseth N J, Thunold K, Selvig K A 2001 Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. European Journal of Orthodontics 23: 475–484

Sutcliffe P 1972 A longitudinal study of gingivitis and puberty. Journal of Periodontal Research 7: 52–58

Truchot G 1991 Do multi-bracket orthodontic appliances favor the development of parasites and fungi in the oral environment? Pathological and therapeutic consequences. Orthodontie Française 62: 1019–1024

Wright P S, Clark P, Hardie J M 1985 The prevalence and significance of yeasts in persons wearing complete dentures with soft-lining materials. Journal of Dental Research 64: 122–125