

Bacterial composition in the supragingival plaques of children with and without dental caries

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Abstract

Purpose: The purpose of the present investigation was to determine if the supragingival bacterial composition plaques in children with caries would differ from those found in caries-free controls.

Material and methods: Pooled supragingival plaque samples from the smooth surfaces of teeth were collected from 75 children with caries and 131 children without caries. The plaque samples were analysed for bacterial content by cultures on a series of non-selective and selective media for aerobic, microaerophilic and anaerobic bacteria. Additionally, the specimens of dentine carious lesions were examined. The standard culture procedures and identifications of bacteria were used.

Results: Among 131 children without dental caries, 41 (31.3%) were at preschool age with deciduous teeth and 90 (68.7%) at school age with permanent teeth. Dental plaques of caries-free children revealed 452 strains, out of which 326 (72.1%) were from permanent teeth, 126 (27.9%) – from deciduous teeth ($p=0.0001$). Among 75 children with dental caries, 61 (81.3%) were at preschool age and 14 (18.7%) – at school age. There were 239 strains isolated from supragingival plaques in children with dental caries, 187 (78.2%) – in preschool children, and 52 (21.8%) – in school children ($p<0.05$). From dentine carious lesions in these children, 209 strains were isolated; 164 from preschool children and 45 – from school children ($p<0.05$). Gram-positive bacteria were isolated more frequently than Gram-negative ones ($p=0.0001$) from supragingival plaques both in children with and without dental caries. *Streptococcus* genus bacteria were isolated more often ($p=0.0002$) from the plaques in

school children without dental caries. The proportion (%) of aerobic and anaerobic bacteria was comparable ($p>0.05$) in dental plaques in children with and without dental caries, except for *Veillonella* spp., which were isolated more frequently from dental plaques in school children with dental caries ($p=0.01$).

Conclusions: 1) Generally, there was no statistically significant difference of bacterial species composition isolated from supragingival plaques in children with deciduous and permanent dental caries and caries-free children. 2) There was no difference between bacterial composition in dentine carious lesions of deciduous teeth and permanent teeth as compared to supragingival plaques in these children (except for *Neisseria* spp., *Peptostreptococcus* spp.).

Key words: bacterial composition, supragingival plaques, dentine carious lesions, deciduous teeth, permanent teeth, caries-free children.

Introduction

Dental plaque is a complex microbial community growing as a biofilm on enamel surfaces. The aetiology of both dental caries (tooth decay) and various forms of periodontal disease has long been recognized to be related to bacterial accumulations and plaque composition. Despite extensive analysis of plaque samples from healthy and diseased subjects as well as data derived from gnotobiotic and germ-free animal experiments, no single microbe has been identified which satisfies Koch's postulates for an infectious agent in either caries or periodontitis [1-5]. Most recent evidence suggests that both diseases have a multibacterial aetiology and therefore it is important to gain insight into the total bacterial composition of dental plaque [2-4]. It has been found that many of early microbial colonizers of human dental plaque are of great importance in the succession stages of biofilm formation and its overall effect on the oral health of the host [4].

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Table 1. Aerobic and anaerobic bacteria in supragingival plaques and carious lesions of children

Bacteria	Specimens (No. of children)	Supragingival plaques			Carious lesions (75)
		Caries (75)	Caries-free (131)	Total (206)	
I. Aerobic bacteria		150	291	441	125
1. Gram-positive		72	148	220	61
Gram-positive cocci		72	148	220	61
<i>Streptococcus</i> spp.		*47	*102	149	37
<i>Gemella morbillorum</i>		0	1	1	1
<i>Staphylococcus</i> spp.		24	41	65	22
<i>Micrococcus</i> spp.		1	4	5	1
2. Gram-negative		78	143	221	64
Gram-negative cocci		59	112	171	47
<i>Neisseria</i> spp.		59	112	171	47
Gram-negative bacilli		19	31	50	17
<i>Haemophilus</i> spp.		12	27	39	12
<i>Enterobacter aerogenes</i>		1	4	5	2
<i>Escherichia coli</i>		*5	*0	5	2
<i>Pseudomonas aeruginosa</i>		1	0	1	1
II. Anaerobic bacteria		89	161	250	84
1. Gram-positive		73	120	193	64
Gram-positive cocci		68	110	178	57
<i>Peptococcus</i> spp.		31	44	75	29
<i>Peptostreptococcus</i> spp.		37	66	103	28
Gram-positive bacilli		5	10	15	7
<i>Actinomyces</i> spp.		0	6	6	0
<i>Bifidobacterium</i> spp.		4	2	6	3
<i>Eubacterium lentum</i>		1	2	3	3
<i>Lactobacillus</i> spp.		0	0	0	1
2. Gram-negative		16	41	57	20
Gram-negative cocci		16	38	54	20
<i>Veillonella</i> spp.		16	38	54	20
Gram-negative bacilli		0	3	3	0
<i>Fusobacterium</i> spp.		0	1	1	0
<i>Prevotella oralis</i>		0	1	1	0
<i>Bacteroides eggerthii</i>		0	1	1	0
Total		239	452	691	209
Gram-positive		145	268	413	125
Gram-negative		94	184	278	84

* p<0.05

The purpose of the present investigation was to determine if the supragingival bacterial plaques in children with caries would differ from those found in caries-free controls.

Material and methods

Pooled supragingival plaque samples from the smooth surface of teeth were collected from 75 children with dental caries and 131 children without caries. The plaque samples were analysed for bacterial content by cultures on a series of non-selective and selective media for aerobic, microaerophilic and anaerobic bacteria. Additionally, the specimens of dentine carious lesions were examined. The standard culture procedures and identifications of bacteria with commercial kits (API Staph, API Strep, API NH, API Coryne, API 20E, API 20NE, API A) were used [3,5,6].

The chi-squared test was used to analyse the differences between the isolation frequencies (shown as percentages or number). Groups were considered significantly different from each other if $P \leq 0.05$.

The approval of the local Ethics Committee was obtained prior to the study.

Results

This study carried out in 206 children (96 males and 110 females) aged 4-18. Of which divided in two groups such as: 102 preschool children aged 4-7 (mean age =5.5 years) and 104 school children (52: 12-year-old and 52: 18-year-old). These children attended to preschool and school in Bialystok.

Caries prevalence were diagnosed visually and only lesions accompanied by enamel loss or cavities involving the dentin of

Table 2. Isolation frequency (%) of bacterial species in supragingival plaques of children without caries

Bacterial species (No. of strains)	Preschool children (n=41)	School children (n=90)	Total (n=131)
Aerobic bacteria			
<i>Streptococcus</i> spp. (102)	*58.5	*86.7	77.9
<i>S. intermedius</i> (3)	4.9	1.1	2.3
<i>S. mitis</i> (22)	17.1	16.7	16.8
<i>S. oralis</i> (12)	7.3	10.0	9.2
<i>S. salivarius</i> (24)	*7.3	*23.3	18.3
<i>S. sanguis</i> (7)	2.4	6.7	5.3
<i>S. vestibularis</i> (34)	19.5	28.9	25.9
<i>Gemella morbillorum</i> (1)	0	1.1	0.8
<i>Staphylococcus</i> spp. (41)	34.1	30.0	31.3
<i>S. aureus</i> (23)	24.4	14.4	17.6
<i>S. capitis</i> (1)	0	1.1	0.8
<i>S. epidermidis</i> (11)	9.8	7.8	8.4
<i>S. haemolyticus</i> (1)	0	1.1	0.8
<i>S. hominis</i> (3)	0	3.3	2.3
<i>S. warneri</i> (2)	0	2.2	1.5
<i>Micrococcus</i> spp. (4)	0	4.4	3.1
<i>Neisseria</i> spp. (112)	85.4	85.6	85.5
<i>N. flavescens</i> (19)	7.3	17.8	14.5
<i>N. mucosa</i> (23)	19.5	16.7	17.6
<i>N. sicca</i> (37)	26.8	28.9	28.2
<i>N. subflava</i> (33)	31.7	22.2	25.2
<i>Haemophilus</i> spp. (27)	12.2	24.4	20.6
<i>H. influenzae</i> (2)	0	2.2	1.5
<i>H. parainfluenzae</i> (25)	12.2	22.2	19.1
<i>Enterobacter aerogenes</i> (4)	2.4	3.3	3.1
Anaerobic bacteria			
<i>Actinomyces naeslundii</i> (6)	4.9	4.4	4.6
<i>Bifidobacterium</i> spp. (2)	2.4	1.1	1.5
<i>Eubacterium lentum</i> (2)	2.4	1.1	1.5
<i>Peptococcus</i> spp. (44)	34.1	33.3	33.6
<i>Peptostreptococcus</i> spp. (66)	48.8	51.1	50.4
<i>Veillonella</i> spp. (38)	21.9	32.2	29.0
<i>Fusobacterium</i> spp. (1)	0	1.1	0.8
<i>Prevotella oralis</i> (1)	0	1.1	0.8
<i>Bacteroides eggerthii</i> (1)	0	1.1	0.8

* p<0.05

the primary teeth or the secondary teeth were considered. The labial, buccal and lingual tooth surfaces were inspected visually for the presence of dental plaque, without using disclosing solution. Children with visible plaque on one or more tooth surfaces were categorized as positive (visible plaque) as well as visible caries. Pooled supragingival plaque and samples of dentine carious lesions were collected and examined bacteriologically. This results are presented in *Tab. 1*.

Among a total of 206 examined children 131 (63.6%) were without dental caries and in 75 (36.4%) caries were found. Dental plaques of caries-free children revealed 452 strains belonged to 16 genera of 7 aerobic and 9 anaerobic bacteria. From dental plaques of children with caries a total 239 strains were isolated, 8 aerobic and 5 anaerobic genera. In addition, from 75 samples of dentine carious lesions 209 strains were detected of which 9 aerobic and 6 anaerobic genera of bacteria (*Tab. 1*). Gram-positive bacteria were isolated more frequently than Gram-negative

ones (p=0.0001) from supragingival plaques both in children with and without dental caries as well as from carious lesions.

Out of 131 children without dental caries, 41 (31.3%) were at preschool age with deciduous teeth and early mixed dentition and 90 (68.7%) at school age with permanent teeth (*Tab. 2*). A total of 326 (72.1%) bacterial strains (29 species) were from permanent teeth and 126 (27.9%) (20 species) – from deciduous teeth (p=0.0001). *Streptococcus* genus bacteria and *Streptococcus salivarius* species were isolated more often (p<0.05) from the plaques in school children without dental caries (*Tab. 2*).

Among 75 children with dental caries, 61 (81.3%) were at preschool age and 14 (18.7%) – at school age (*Tab. 3*). There were 239 strains isolated from supragingival plaques in children with dental caries, 187 (78.2%) – in preschool children (23 species), and 52 (21.8%) – in school children (17 species) (p<0.05). From dentine carious lesions in these children, 209 strains were isolated; 164 (78.5%) from preschool children (29 species) and

Table 3. Isolation frequency (%) of bacterial species in supragingival plaques and dental carious lesions of children with caries

Bacterial species (No. of strains)	Preschool children (n=61)		School children (n=14)		Total (n=75)	
	Plaque	Cariou lesion	Plaque	Cariou lesion	Plaque	Cariou lesion
Aerobic bacteria						
<i>Streptococcus</i> spp. (84)	59.0	45.9	78.6	64.3	62.7	49.3
<i>S. intermedius</i> (2)	0	1.6	0	7.1	0	2.7
<i>S. mitis</i> (21)	13.1	13.1	21.4	14.3	14.7	13.3
<i>S. mutans</i> (2)	1.6	1.6	0	0	1.3	1.3
<i>S. oralis</i> (15)	8.2	9.8	14.3	14.3	9.3	10.7
<i>S. salivarius</i> (13)	9.8	6.6	7.1	14.3	9.3	8.0
<i>S. sanguis</i> (9)	8.2	6.6	0	0	6.7	5.3
<i>S. vestibularis</i> (22)	18.0	6.6	35.7	14.3	*21.3	*8.0
<i>Gemella morbillorum</i> (1)	0	1.6	0	0	0	1.3
<i>Staphylococcus</i> spp. (46)	34.4	29.5	21.4	28.6	32.0	29.3
<i>S. aureus</i> (19)	16.4	14.7	0	0	13.3	12.0
<i>S. caprae</i> (1)	0	1.6	0	0	0	1.3
<i>S. cohnii</i> (1)	0	1.6	0	0	0	1.3
<i>S. epidermidis</i> (11)	11.5	4.9	7.1	0	10.7	4.0
<i>S. hominis</i> (5)	1.6	1.6	7.1	14.3	2.7	4.0
<i>S. simulans</i> (1)	0	0	0	7.1	0	1.3
<i>S. warneri</i> (8)	4.9	4.9	7.1	7.1	5.3	5.3
<i>Micrococcus</i> spp.(2)	0	0	7.1	7.1	1.3	1.3
<i>Neisseria</i> spp. (106)	*77.0	*59.0	85.7	78.6	*78.7	*62.7
<i>N. flavescens</i> (13)	11.5	4.9	7.1	14.3	10.7	6.7
<i>N. mucosa</i> (17)	9.8	14.7	14.3	0	10.7	12.0
<i>N. sicca</i> (36)	22.9	16.4	50.0	35.7	28.0	20.0
<i>N. subflava</i> (40)	32.8	22.9	14.3	28.6	29.3	24.0
<i>Haemophilus</i> spp. (24)	11.5	14.7	35.7	21.4	16.0	16.0
<i>H. influenzae</i> (1)	0	1.6	0	0	0	1.3
<i>H. parainfluenzae</i> (23)	11.5	13.1	35.7	21.4	16.0	14.7
<i>Enterobacter aerogenes</i> (3)	1.6	3.3	0	0	1.3	2.7
<i>Escherichia coli</i> (7)	8.2	3.3	0	0	6.7	2.7
<i>Pseudomonas aeruginosa</i> (2)	1.6	1.6	0	0	1.3	1.3
Anaerobic bacteria						
<i>Bifidobacterium</i> spp. (7)	3.3	3.3	14.3	7.1	5.3	4.0
<i>Eubacterium lentum</i> (4)	1.6	4.9	0	0	1.3	4.0
<i>Lactobacillus</i> spp. (1)	0	1.6	0	0	0	1.3
<i>Peptococcus</i> spp. (60)	44.3	**45.9	28.6	**7.1	41.3	38.7
<i>Peptostreptococcus</i> spp. (65)	*49.2	**31.1	50.0	**64.3	49.3	37.3
<i>Veillonella</i> spp. (36)	**14.7	22.9	**50.0	42.9	21.3	26.7

* p<0.05; ** p<0.05

45 (21.5%) – from school children (17 species) (p<0.05). There was no difference between bacterial composition in dentine carious lesions of preschool children and school children as compared to supragingival plaques in these children, except for *Neisseria* spp. and *Peptostreptococcus* spp. (Tab. 3). These genera more often were detected in dental plaques than carious lesions of preschool children (p<0.05). *Peptostreptococcus* spp. more frequently was also isolated from carious lesions of school children than preschool children.

Discussion

According to the results of the present study it is concluded that, there was no statistically significant difference of bacterial

species composition isolated from supragingival plaques in children with deciduous and permanent dental caries and caries-free children. There was also no difference between bacterial composition in dentine carious lesions of deciduous teeth and permanent teeth as compared to supragingival plaques in these children, except for *Neisseria* spp. and *Peptostreptococcus* spp. These bacteria more often were isolated from supragingival plaque of preschool children (77.0% and 49.2%, respectively) than from carious lesions (59.0% and 31.1%) (p<0.05). *Veillonella* spp. was isolated significant frequently from dental plaques in school children with dental caries (7/14; 50.0%) than preschool children (9/61; 14.7%) (p=0.01) while *Peptococcus* spp. was more frequent in carious lesions of preschool children (28/61; 45.9%) than school children (1/14; 7.1%) (p=0.017).

Mutans streptococci and *Lactobacillus* spp. which are the main aetiological agents of dental caries in humans [1-3,7] were observed only sporadically in preschool children. *Actinomyces* spp. was detected only in dental plaque of children without caries. The proportion of cariogenic bacteria is about 10% and was comparable ($p > 0.05$) in dental plaques in children with and without dental caries in both the preschool and school children as well as in carious lesions of primary and permanent teeth.

Gram-positive bacilli other than *Lactobacillus* spp. and *Actinomyces* spp. accounted for about 60% of the total Gram-positive anaerobic rods. Some species belonging to *Bifidobacterium* spp. and *Eubacterium lentum* are also found to be associated with periodontal disease and could frequently be isolated from carious lesions [1].

Most, if not all, forms of dental decay are chronic bacterial infections due to the dominance in the plaques of aciduric bacterial species such as the mutans streptococci, lactobacilli and *Actinomyces* spp. [1-7]. The results obtained by Aamdal et al. [8] do not readily support the traditional concept of caries formation according to hypothesis that root caries is the results of acid formation by acidogenic microorganisms. Authors was not detected difference in microbial composition of dental plaque and difference in plaque pH response on sound and carious root surfaces. The plaque pH response was more pronounced in the maxilla than in the mandible for both sound and carious sites. The pH response to sucrose was the same regardless of the presence or absence of mutans streptococci [8].

Despite these finding, neither *Streptococcus* spp. nor *Lactobacillus* spp. appear to be predominant pathogens in root caries, as others have shown contradictory findings [9]. It is appears that both these bacterial groups play an important role in the initiation and development of caries together with a variety of other oral microbiota, as shown by our own results and by others [10].

Our study showed that some bacterial species which are putative respiratory pathogens [11] (e.g. *S. aureus*, *H. influenzae*, *H. parainfluenzae*, *P. aeruginosa* and *E. coli*) had colonized the supragingival plaques of preschool and school children as well as children with and without caries. The isolation proportion was about 12% and was comparable, also in the samples of carious lesions.

In conclusions, culturing organisms remains an important tool for the detection of bacteria from dental plaque and other site of oral cavity. Cultured microorganisms are required for antibiotic resistance data and for elucidation of virulence mechanism. However, cultured bacteria may rapidly alter their phenotypic characteristics in vitro, and 50% of oral microorganisms have not yet been cultured [12,13].

It will also be important to control the oral microflora for systemic reasons since strong links are being established between focal infection of oral origin and a range of systemic diseases including coronary heart disease, gastrointestinal disorders and low birth weight, apart from severe overt systemic infection [13-15]. These developments are derived from an improved understanding of the ecological nature of the microbial biofilm that is dental plaque, and of its interactions with human host [15,16].

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