The Bacteriology of the Supragingival Plaque of Child Dental Patients in Kuwait

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Key Words

Supragingival plaque · Microbiology · Children · Kuwait

Abstract

Objective: To investigate the bacterial flora of the gingival plaques of pediatric dental patients in order to establish baseline data for future studies on the complex microbiota of the oral cavity of this group of child dental patients. **Subjects and Methods:** Forty subjects, aged 3–12 years, were enrolled and investigated after informed consent was obtained. During the patients' first visit to the dental clinic supragingival plaque samples were collected from the gingival area of buccal and lingual tooth surfaces of all primary and permanent molar teeth using sterile curettes. Serially diluted samples were inoculated onto a set of selective and nonselective media and then incubated at the appropriate atmospheric conditions and temperatures. Representative colonies of isolates were identified by standard methods, including hemolytic characteristics on blood agar and profiling on API 20S and API 20A as appropriate. **Results:** A total of 183 isolates (123 aerobes and 60 anaerobes) were recovered from the 40 patients with a mean count of 3 per sample of aerobes compared to 1.5 per sample for the anaerobes. *Streptococcus sanguis* was the predominant

aerobic species (23.6%) isolated from 29 (72.5%) of the 40 patients, followed by *Streptococcus mitis*, 19.5 and 60%, *Streptococcus salivarius*, 17.1 and 52.5% and *Streptococcus mutans*, 17.1 and 52.5%. The frequency of isolation of anaerobic bacteria was *Prevotella* spp. 50% from 30 (75%) of the 40 patients, *Fusobacterium* spp., 18.3 and 27.5%, *Bacteroides* spp., *Porphyromonas* spp. and *Peptostreptococcus* spp., 6.7 and 10%, 6.7 and 10%, and 5 and 7.5%, respectively. *Prevotella intermedia* was the single most common species. **Conclusion:** The results show that there were more aerobic/facultative anaerobic bacteria than the obligate anaerobes in the dental plaque of the pediatric dental patients in Kuwait. The high prevalence of the pioneering streptococci as well as black-pigmented *Prevotella* spp. and *Fusobacterium* spp. indicates that the dental plaques of the children were in the developing stage, a precursor to the development of periodontal diseases.

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Introduction

The oral cavity provides ecological niches to a large number of diverse microbiota – bacteria, fungi, protozoa and viruses – that form the normal resident flora [1]. Over

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300 different species exist in the human oral cavity [2]. The members of this flora exist in a harmonious relationship with the host. However, once this homeostasis is lost, a significant number of these microorganisms can become pathogenic giving rise to opportunistic infections which include mucosal infections, dental caries and periodontal diseases [3–5].

Microbial plaque, which lies in close proximity to gingival tissue, is an important ecological factor instigating gingival inflammation and, in some cases, subsequent loss of the periodontal tissue [1]. Therefore, to evaluate the flora responsible for adverse dental infections, the bacterial population of the dental plaque needs to be examined. Enumeration of microorganisms forming the plaque is important so as to obviate the perpetuation of plaque of dental caries and periodontitis, and also in formulating an informed evidence-based policy on a proper antimicrobial regimen for the treatment of dental infections.

In this study, we present a preliminary report of a prospective study designed to enumerate the distribution of the bacterial population of the supragingival plaques of Kuwaiti child dental patients.

Subjects and Methods

Forty subjects, aged 3–12 years, who agreed to participate in the study after careful explanation of the objectives to their parents whose consents were obtained, were enrolled and investigated. The supragingival plaque was sampled from the child patients on their first visit to the dental clinic by one of us (N.O.S.). Plaque samples were collected from the gingival area of buccal and lingual tooth surfaces of all primary and permanent molar teeth using sterile curettes. The scrapings were immediately dispersed into 0.3 ml prerduced transport fluid (thioglycollate broth; Oxoid, Basingstoke, UK) and transported by the same clinician without delay to the Anaerobe Laboratory, Department of Microbiology, Faculty of Medicine where all the laboratory investigations were conducted.

In the laboratory, each sample was sonicated and diluted serially (10-fold) in freshly prepared prerduced thioglycollate broth (Oxoid). Then a 0.01-ml aliquot of each dilution was inoculated onto a set of selective and nonselective media. For the enumeration of the oral streptococci, Mitis-Salivarius agar (Difco Laboratories, Detroit, Mich., USA) supplemented with 0.1% (v/v), and Chapman Tellurite solution with and without bacitracin 0.2 U/ml (Sigma, St. Louis, Mo., USA) was used [6]. The hemolytic pattern of the streptococcal species was studied using brain heart infusion agar (BHIA; Oxoid) supplemented with 5% human blood. For the isolation of anaerobes, blood agar (Oxoid) supplemented with gentamicin 75 µg/ml and fastidious anaerobic agar supplemented with hemin 1 µg/ml (Sigma), menadione 1 µg/ml (Sigma), gentamicin 75 µg/ml and 5% human blood were used; they were freshly prepared daily and prerduced for 2 h before use.

The plates were incubated at the appropriate atmospheric conditions and temperatures. For aerobic incubations, the plates were held

Table 1. The quantitative and numerical counts of the aerobic and anaerobic flora of the supragingival plaque of Kuwaiti child dental patients

Quantitation	Aerobes	Anaerobes
<i>Total count, cfu/ml</i>		
Mean	5×10^4	3.9×10^5
Range	2×10^2 – 2.8×10^5	1×10^2 – 2.4×10^6
<i>Number of isolates</i>		
Mean	3	1.5
Range	0–6	0–5
cfu = Colony-forming unit.		

in air at 37 °C for 24 h and extended for 48 h if there was no growth. The inoculated plates meant for anaerobic incubation were placed in anaerobic jars (Oxoid) containing CO₂ 10%, H₂ 90%, generated by anaerobic gas generating kit (Oxoid), and then placed in the incubator at 37 °C for 48 h and 5 days. Quantitative bacterial count of anaerobes and aerobes was made after the appropriate incubation periods. Representative colonies of isolates were identified by standard methods [7].

Streptococci were grouped initially by their hemolytic characteristics on blood agar and also profiling on API 20S (BioMérieux, Hazelwood, Mo., USA). Anaerobes were identified by the lack of growth under aerobic culture, initial susceptibility to metronidazole disk (5 µg), colonial and gram strain morphology, and confirmed by specific reactions in API 20A (BioMérieux).

Results

All the samples from the 40 children were processed for the enumeration of the prominent bacterial flora in the supragingival plaque quantitatively and qualitatively. A total of 183 isolates were recovered from the patients. Table 1 shows the viable bacterial counts and the number of isolates per specimen. In terms of quantitative counts the strict anaerobes were more prevalent than the aerobes by about 1 log₁₀. However, the aerobes were numerically more prevalent in the plaques with a mean count of 3 per sample compared to 1.5 per sample for the anaerobes, with ranges of 0–6 and 0–5 for aerobes and anaerobes, respectively.

Table 2 shows that a total of 123 isolates were recovered, together with the proportion and frequency of isolation of the different *Streptococcus* and *Lactobacillus* species prevalent in the dental plaques. *Streptococcus sanguis* was the predominant species, representing 23.6% of all the facultative aerobic flora and it was isolated from 29

out of 40 patients (72.5%). It was followed by *Streptococcus mitis*, 19.5% and from 24 out of 40 (60%), *Streptococcus salivarius*, 17.1% and from 21 of 40 (52.5%) and *Streptococcus mutans*, 17.1% and from 21 out of 40 (52.5%). The other streptococci were isolated in relatively low proportions of patients. *Lactobacillus* species were isolated in only 3 of the 40 patients (7.5%).

A total of 60 anaerobic bacteria were isolated from the 40 patients. The species, proportion and frequency of isolation of obligate anaerobic bacteria are shown in table 3. *Prevotella* spp. were the commonest anaerobe isolated, representing 50% of the total anaerobes; it was present in the gingival plaque of 30 (75%) out of 40 patients. The next in the ranking order by a far margin was the group of *Fusobacterium* spp., which represented 18.3% of the anaerobes and isolated from 27.5% of the patients. Other notable anaerobes of clinical importance such as *Bacteroides* spp., *Porphyromonas* spp. and *Peptostreptococcus* spp. represented relatively low proportions of the anaerobic flora (6.7, 6.7 and 5%, respectively) and were also present in a low number of patients, 10, 10 and 7.5% respectively. Detailed analysis of the *Prevotella* species is shown in table 4, which reveals that *Prevotella intermedia* was the predominant species, representing 36.7% of the *Prevotella* spp., followed by the nonpigmented *Prevotella oralis* 26.6% and *Prevotella melaninogenica* 23.3%.

Discussion

Plaque is generally regarded as a macrohabitat with many microenvironments attributable to the continuous change in the ecological parameters. The progression to and composition of plaque follows a definite pattern of bacterial colonization. Colonization by the so-called pioneer species (*S. mitis*, *Streptococcus oralis*, *S. sanguis*) is followed by a gradual increase in the complexity of the microflora culminating in the establishment of a highly diverse group comprising many spiral, filamentous and obligate anaerobic bacteria [8].

In this study, we established baseline data on the bacterial flora of the supragingival plaque of pediatric dental patients in Kuwait. The data demonstrate a relatively high prevalence of *S. sanguis* and *Prevotella* spp. at this site. Of course, the relatively high isolation rate of *S. sanguis*, *S. mitis* and *S. mutans* is noteworthy because *S. sanguis* and *S. mitis* comprise a significant part of the pioneer species that contribute to plaque formation usually associated with healthy plaques. The presence of a large number of *S. mutans* is also worth mentioning because this may in

Table 2. Proportion and frequency of isolation of different aerobes in the dental plaque of 40 subjects

Bacterial isolates	Isolates	Proportion of total, %	Frequency of isolation, %
<i>Streptococcus sanguis</i>	29	23.6	72.5
<i>Streptococcus mitis</i>	24	19.5	60
<i>Streptococcus salivarius</i>	21	17.1	52.5
<i>Streptococcus mutans</i>	21	17.1	52.5
<i>Streptococcus oralis</i>	8	6.5	20
<i>Streptococcus constellatus</i>	4	3.3	10
<i>Streptococcus acidominimus</i>	4	3.3	10
<i>Streptococcus intermedius</i>	3	2.4	7.5
<i>Streptococcus bovis</i>	2	1.6	5.0
<i>Streptococcus equinus</i>	2	1.6	5.0
<i>Streptococcus adjacens</i>	2	1.6	5.0
<i>Lactobacillus</i> spp.	3	2.4	7.5
Total	123	100	

Table 3. Proportion and frequency of isolation of different anaerobes in dental plaque of 40 subjects

Bacterial isolates	Isolates	Proportion of total, %	Frequency of isolation, %
<i>Prevotella</i> spp.	30	50	75
<i>Fusobacterium</i> spp.	11	18.3	27.5
<i>Bacteroides</i> spp.	4	6.7	10
<i>Porphyromonas</i> spp.	4	6.7	10
<i>Veillonella</i> spp.	4	6.7	10
<i>Peptostreptococcus</i> spp.	3	5.0	7.5
<i>Eubacterium</i> spp.	2	3.3	5.0
<i>Capnocytophaga</i> spp. ¹	1	1.7	2.5
<i>Bifidobacterium</i> spp.	1	1.7	2.5

¹ Not a strict anaerobe.

Table 4. Frequency of isolation of the different species that make up the *Prevotella* spp. in the dental plaque of subjects

<i>Prevotella</i> spp.	Frequency of isolation from patients (n = 30)	
	n	%
<i>Prevotella intermedia</i>	11	36.7
<i>Prevotella oralis</i>	8	26.7
<i>Prevotella melaninogenica</i>	7	23.3
<i>Prevotella disiens</i>	3	10
<i>Prevotella bivia</i>	1	3.3

fact signify a shift to a more cariogenic plaque; this is supported by the fact that all the children who participated in this survey had caries with a mean decayed filled teeth (DFT) of 6.5 ± 2.1 .

In a healthy gingival environment, the predominant microorganisms are gram-positive cocci and rods, and during the maturation of plaque there is gradual shift to a complex community with flora that mainly comprises gram-negative rods including the filaments and the fusiforms [8]. This process plays a predominant role in the progression to periodontitis and gingivitis. Our study demonstrates a high isolation rate of black-pigmented *Prevotella* spp., followed by the *Fusobacterium* spp., which, conceivably, suggests the attainment of the developing stage of the plaque in most of our subjects (table 3). These findings are in accord with many previous reports that found as many as 61% of children aged 5–7 years harboring black-pigmented gram-negative anaerobes [9–11]. *P. intermedia* was the predominant anaerobic species (table 4), a finding which further supports the fact that the plaque covering the teeth of children in Kuwait are in the developing stage. This is a precursor to progression to periodontitis. This is buttressed by the fact that *P. intermedia* has been known to increase the activity of degradative enzymes under certain conditions, and supports the progression to periodontitis [12].

Another important member of the supragingival flora of the mouth of the pediatric subjects is *Fusobacterium* spp. These were also isolated from a relatively high number of the subjects and in high proportion after *P. intermedia* (table 4). These organisms have been shown to play an important role in the formation of dental plaque through their ability to extensively coaggregate with many other oral microorganisms [13–17]. Since supragingival plaque can harbor putative periopathogens, as demonstrated in this study, it is conceivable to speculate that it probably plays an important role as a reservoir for such organisms and consequently may lead to the spread to, or reinfection of, subgingival sites [18]. It is therefore reasonable to assume that if the development or maturation of the plaque is undisturbed, or not prevented, it may eventually provide a hospitable milieu for a number of periodontal pathogens.

However, many reports have shown that severe gingivitis and periodontal disease are very rare in healthy, non-medically compromised children. This has led to the conclusion that the prevalence and severity of gingival diseases may be related to dietary factors, or an age-dependent reactivity to plaque accumulation and/or genetic predisposition and not the mere plaque formation per se.

A few studies in the past demonstrated that with a similar amount of plaque, healthy children tended to have less gingival inflammation than older individuals [19, 20]. Differences in the immunological response to the periodontic pattern of bacterial flora are a possible explanation for this observation [21–23].

Conclusion

The results of the present study showed that the profile of the common oral bacteria is essentially in agreement with the findings in similar settings in other geographical parts of the world and that there are more aerobic/facultative anaerobic bacteria than obligate anaerobes in the dental plaque of the pediatric dental patients in Kuwait. The high prevalence of the pioneering streptococci as well as black-pigmented *Prevotella* spp. and *Fusobacterium* spp. indicates that the dental plaques of our children are in the developing stage, a precursor to the development of periodontal diseases.

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