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Oral Microbiota of Children in a School-based Dental Clinic

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Abstract

Objectives—Dental caries disproportionately affects disadvantaged subjects. This study hypothesized that there were greater caries extent and higher levels of caries-associated and anaerobic subgingival bacterial species in oral samples of Hispanic and immigrant children compared with non-Hispanic and US born children.

Methods—Children from a school-based dental clinic serving a community with a large Hispanic component were examined, and the extent of caries was recorded. Microbial samples were taken from teeth and the tongues of children. Samples were analyzed using DNA probes to 18 oral bacterial species.

Results—Seventy five children were examined. Extent of caries increased with child age in immigrant, but not in US born or Hispanic children. There were no differences in the microbiota based on ethnicity or whether the child was born in US or not. There was a higher species detection frequency from teeth than tongue samples. Levels of *Streptococcus mutans* and other *Streptococcus* species increased with caries extent. *Prevotella intermedia*, *Tannerella forsythia* and *Selenomonas* species were detected at low levels in these children.

Conclusions—We conclude that, while there was a high rate of dental caries in disadvantaged school children, there were no differences in the caries-associated microbiota, including *S. mutans*, based on ethnicity or immigration status. Furthermore, while anaerobic subgingival, periodontal pathogens were also detected in children, there was no difference in species detection based on ethnicity or immigration status. Increased levels of streptococci, including *S. mutans*, however, were detected with high caries levels. This suggested that while it is beneficial to target preventive and treatment programs to disadvantaged populations, there is likely no additional benefit to focus on subgroups within a population already at high risk for dental disease.

Keywords

Dental Caries; School Children; Hispanic; *Streptococcus mutans*; *Prevotella intermedia*

1 Introduction

Dental caries, gingivitis and periodontitis can negatively impact oral and general health. These dental infections disproportionately affect disadvantaged populations including Hispanic [1–3] and immigrant populations [4–6]. Since bacteria associated with caries, gingivitis and periodontitis can be detected in childhood [7–15], increased infection by dental pathogens in Hispanic and immigrant may suggest that dental public health measures, including targeted preventive measures, would reduce the dental disease in these high risk populations.

Dental caries leads to loss of tooth structure requiring restorations, which can be costly. When left untreated in children, spread of the caries infection from the primary to the secondary dentition [16] leads to increased treatment needs. Severe caries can lead to endodontic infections, with pain and abscess formation, which in children can lead to impaired speech, nutritional deficiencies and loss of school days. Periodontal diseases, including gingivitis, can be detected in children and adolescents particularly in mixed and permanent dentitions [17]. The impact of periodontitis in adolescence is of particular concern because it can lead to loss of tooth function and may increase risk of periodontitis-associated systemic consequences [18].

The primary pathogens for childhood caries include *Streptococcus mutans* and *Streptococcus sobrinus*, acidogenic non-mutans *Streptococcus* [19], and *Lactobacillus* species. Caries-associated species have been detected in children using culture, particularly selective culture for *S. mutans* and *Lactobacillus* species [11,20,21], and molecular methods including PCR [22,23] and using DNA probe [7,9,14,15] analyses. Subgingival species, including the periodontal pathogens *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, have also been detected in children using culture [8,10], and molecular methods [12,14]. Culture and molecular studies have revealed diversity in species and phylotypes in children that is comparable to that detected in adults. Few studies, however, have evaluated whether there are microbial differences based on Hispanic ethnicity or immigrant status.

In this study we examined children in a school-based dental clinic serving the dental needs of a community with a large low income, Hispanic population as part of our studies of the oral microbiota of children from disadvantaged populations. We hypothesized that differences in disease experience in Hispanic and immigrant children would be reflected in different microbiotas. The purpose of this study thus was to determine if there were microbial differences associated with child ethnicity (Hispanic or not), and whether the child was born in the United States of America (US). Differences in disease extent and the oral microbiota based on ethnicity and immigration status would suggest that treatment and public health-based preventive programs might need to be targeted to subsets of the population at greatest risk of infection.

2 Methods

2.1 Study population, microbial sampling and analysis

Children were recruited from the population of children seeking care at a school-based dental clinic which serves a financially disadvantaged, largely Hispanic population. Children were selected as they presented for treatment and agreed to microbial sampling. Approval to use samples for research was obtained from the Office of the Institutional Review Board, Boston University, and from The Forsyth Institute under the approval to handle samples for the Northeast Center for Research to Reduce Oral Health Disparities. Child age, ethnicity (Hispanic/Latino or not) and whether or not the child was born in the US or Canada was by self or parent report. Samples were taken using a cleoid discoid carver from three intraoral sites, molar occlusal surfaces, interproximal molar tooth surfaces, and the dorsum of the tongue.

Samples were put in 150 µl Tris-EDTA (TE) buffer and 150 µl 0.1N NaOH was added in the dental clinic to stabilize the microbial DNA.

2.2 Microbial assays

Samples were analyzed using whole genomic probes in a checkerboard assay as has been described for periodontal sites in adults [24] and in young children [14]. Briefly, samples were placed immediately in 0.1 ml of TE buffer (10 mM Tris, 1mM EDTA, pH 7.6), by carefully shaking the carver in the liquid to remove all plaque collected, followed by addition of 0.1 ml of 0.5 M NaOH was added stabilize DNA. Samples were transported to the microbiology laboratory. Samples were denatured, neutralized, and fixed onto a nylon membrane by ultraviolet (UV) irradiation. Two lanes in each nylon membrane were used for a mixed DNA standard of cells from all probe species at 10^5 and 10^6 cell equivalents. Whole chromosomal DNA probes were prepared using DNA extracted from reference strains of 18 oral species selected to represent caries, gingivitis and periodontitis-associated bacteria. Digoxigenin-labeled, whole chromosomal DNA probes were prepared by random primer labeling using the Genius™ 2 kit (Boehringer Mannheim, Indianapolis, IN). The membranes containing the samples and standards were hybridized with the digoxigenin-labeled probes to target species. Anti-digoxigenin antibodies, covalently bound to alkaline phosphatase, were used to visualize the final product with standard chemiluminescence. Signals were detected using a Storm Fluorimager system (Molecular Dynamics, Sunnyvale, CA) and subsequently converted to absolute bacterial counts by comparison with the DNA standards on the membrane. Signals were detected using a Storm Fluorimager (Molecular Dynamics, Sunnyvale, CA), and reactions enumerated [24]. Signals were converted to absolute counts by comparison with the 10^5 and 10^6 standards on each membrane. The sensitivity of the assay was adjusted to 10^4 cells by setting readings below 10^4 to zero [24]. In this assay, for pure cultures, there were minimal cross reactions within genera, which for *Streptococcus* species did not exceed 100:1 for homologous: heterologous species. Only probes that showed at least this level of specificity in test runs were used. In use, there were possible cross reactions within *Streptococcus*, *Actinomyces*, and *Capnocytophaga* species, *Fusobacterium nucleatum* subspecies, and between *Prevotella intermedia* and *P. nigrescens*.

Data Analysis—The extent of caries was calculated by summing the number of decayed and filled deciduous (dft) and permanent (DFT) teeth for each child. This combined dft with DFT (dft+DFT) score was compared between children by gender by the Kruskal Wallis test. The extent of caries was plotted against age by ethnicity and immigrant status for each child and compared among all children by ethnicity and immigration status using regression analysis and Kruskal Wallis test.

Species detected at 10^5 cells were compared between occlusal, molar interproximal and tongue samples by Chi-square. Species detection frequencies between Hispanic and non-Hispanic, and between US born and immigrant children were compared by Kruskal Wallis test. The microbiota associated with dental caries was compared between mean species levels of children, divided into three groups based on dft+DFT scores in three categories, dft+DFT 0–2, 3–10 and 11–23. Species in these three clinical categories were compared using Kruskal Wallis test. Bonferroni adjustment was made for multiple comparisons.

3 Results

3.1 Population, clinical characteristics

The school dental clinic children sampled (n=75) were aged 4–18 years, 55% were male. Seventy eight percent of children were Hispanic, 32% immigrant (not born in the USA or Canada). The extent of caries (dft+DFT score) was higher in males (p=0.039). Figure 1

illustrates an increase of extent of caries with child age in both Hispanic (1a) and immigrant (1b) populations, but not in non-Hispanic and US born children. There was no difference in age, gender, caries, or increase in caries with age between Hispanic and non-Hispanic children. Immigrant compared with US born children were older (means 10.9 and 8.5 years respectively) and had more caries dft+DFT (10.2 and 7.7 respectively), but these differences were not significant. The increase of caries with age in the immigrant children was greater than non-immigrant children ($p=0.047$). Immigrant Hispanic children ($n=19$) had more caries compared with non-immigrant Hispanic children ($n=23$) ($p=0.041$) when children of all ages were compared, but not when only 6–12 year old children were compared (From Figs 1a and 1b).

3.2 Microbial data

The species detected most frequently were Gram positive *Streptococcus* species, particularly *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus salivarius* and *Streptococcus anginosus* (Figures 2–5). Subgingival (gingivitis and/or periodontitis) species detected included *Capnocytophaga sputigena*, *Prevotella intermedia*, *Selenomonas* species and low levels of the periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*.

3.3 Intraoral sample sites

Not all occlusal and tongue samples of the 75 children yielded sufficient sample for analysis. A comparison between sample sites, based on species detection frequency equivalent to 10^5 cells, from 54 children in whom species were detected in all three occlusal, interproximal molar and tongue, sample sites is in Figure 2. Species were detected more frequently from the occlusal and interproximal tooth sites than from the tongue, but differences were not significant.

3.4 Bacterial species and ethnicity, immigrant status and extent of caries

Microbial comparisons with caries and subject demographics were from interproximal molar samples as all children ($n=75$) had these sites positive than from tongue or occlusal samples. There were no significant differences in species detection frequency between Hispanic and non-Hispanic subjects (Figure 3), or between immigrant and non-immigrant children (data not shown). Several species were detected at higher mean levels with higher dft/DFT scores including the caries-associated species *S. mutans*, *S. sobrinus*, and *Lactobacillus casei*, and the periodontal pathogens *A. actinomycetemcomitans* and *P. intermedia* (Figure 4) but were not significant (after Bonferroni adjustment).

4 Discussion

Children attending a school-based dental clinic, in an underserved city with a predominantly Hispanic population showed high levels of dental caries, which increased with child age. Increase in caries extent with child age is consistent with data in NHANES III [1]. In the study population the highest caries levels were in Hispanic children born outside the US (or Canada), as has previously been observed in Mexican American children and adolescents [1] and in immigrants [5,6]. Caries was higher in males as has been previously observed [25]. Higher caries in the adolescent schoolchildren suggest a need for school based oral health 14: and children in other similar populations in low income communities.

No differences in the microbiota were detected based on Hispanic or immigrant status of children. These findings are consistent with a report from a larger population of adults that did not find significant differences in levels of mutans streptococci and *Lactobacillus* species based on ethnicity (Hispanic, Asian, Black and Caucasian subjects) [26]. This finding suggests that treatment and public health measures may not need to be based on ethnicity or immigrant status of children, but on the observed clinical condition.

Plaque samples from teeth and the tongue were obtained. Since it is often more convenient and efficient to collect samples from the tongue in a non-clinical setting, the results from the two sites were compared to see if tongue samples could be used exclusively. Plaque samples from teeth, however, provided higher microbial detection levels than from the tongue for the selected caries and subgingival species. Lower dental species detection on the tongue was previously observed in young children 18–36 months of age [15] and in children 3–17 years [13]. This finding suggests that teeth provide a better site for microbial screening than the tongue.

None of the tested species were significantly associated with increased levels of caries measured by a combined carious and filled tooth score. The cariogenic species *S. mutans* and *S. sobrinus*, showed modest increased levels with increased caries. The lack of significant associations with caries was probably because most children had caries, and a comparison between caries and caries-free children was not possible. Other potentially cariogenic species, *L. casei* and *Bifidobacterium dentium* were detected at lower levels than the *Streptococcus* species. Species associations with caries particularly for *S. mutans*, *S. sobrinus* and *A. actinomycetemcomitans* were similar to those observed for a larger, mainly non-Hispanic population of pre-school children [27].

Most of the anaerobic subgingival species assayed were also detected at higher levels from increased caries but the detection frequency of periodontal pathogens was low. These frequencies, however, were comparable with a PCR-based study of adolescent school children that harbored *A. actinomycetemcomitans* (15%) and *T. forsythia* (14%) [12], and provide further evidence of colonization of periodontal pathogens in childhood. Whether detection of these species represents an increased risk of periodontitis in adults would require longitudinal clinical and microbiological monitoring. Overall our microbiology findings are comparable with those of the literature describing bacteria detected in children and adolescents.

4.1 Summary and conclusions

As expected, children with extensive caries experienced the highest levels of cariogenic species. Dental caries and the associated microbiota did not differ among Hispanic and non-Hispanic, or between immigrant and US born children in this low income disadvantaged population. Low levels of periodontal pathogens were detected in children. Clinically, these findings support the need for preventive programs and dental care targeting the entire population of low-income, disadvantaged children, irrespective of ethnicity or immigrant status. Further, despite the ease of taking plaque samples from the tongue, especially in non-clinical settings, samples taken from teeth had a greater potential for detecting dental pathogens than tongue samples.

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References

1. Vargas CM, Crall JJ, Schneider DA. Sociodemographic distribution of pediatric dental caries: NHANES III, 1988–1994. *J Am Dent Assoc* 1998;129:1229. [PubMed: 9766104]
2. Flores G, Fuentes-Afflick E, Barbot O, Carter-Pokras O, Claudio L, Lara M, McLaurin JA, Pachter L, Ramos-Gomez FJ, Mendoza F, Valdez RB, Villarruel AM, Zambrana RE, Greenberg R, Weitzman M. The health of Latino children: urgent priorities, unanswered questions, and a research agenda. *JAMA* 2002;288:82. [PubMed: 12090866]
3. Brown LJ, Brunelle JA, Kingman A. Periodontal status in the United States, 1988–1991: prevalence, extent, and demographic variation. *J Dent Res* 1996;75(Spec No):672. [PubMed: 8594091]

4. Locker D, Clarke M, Murray H. Oral health status of Canadian-born and immigrant adolescents in North York, Ontario. *Community Dent Oral Epidemiol* 1998;26:177. [PubMed: 9669596]
5. Maserejian NN, Trachtenberg F, Hayes C, Tavares M. Oral health disparities in children of immigrants: dental caries experience at enrollment and during follow-up in the New England Children's Amalgam Trial. *J Public Health Dent* 2008;68:14. [PubMed: 18179466]
6. Pollick HF, Rice AJ, Echenberg D. Dental health of recent immigrant children in the Newcomer schools, San Francisco. *Am J Public Health* 1987;77:731. [PubMed: 3578623]
7. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, Leys EJ, Paster BJ. Bacteria of dental caries in primary and permanent teeth in children and young adults. *J Clin Microbiol* 2008;46:1407. [PubMed: 18216213]
8. Alaluusua S, Asikainen S. Detection and distribution of *Actinobacillus actinomycetemcomitans* in the primary dentition. *J Periodontol* 1988;59:504. [PubMed: 3171863]
9. Corby PM, Lyons-Weiler J, Bretz WA, Hart TC, Aas JA, Boumenna T, Goss J, Corby AL, Junior HM, Weyant RJ, Paster BJ. Microbial risk indicators of early childhood caries. *J Clin Microbiol* 2005;43:5753. [PubMed: 16272513]
10. Kamma JJ, amanti-Kipioti A, Nakou M, Mitsis FJ. Profile of subgingival microbiota in children with mixed dentition. *Oral Microbiol Immunol* 2000;15:103. [PubMed: 11155173]
11. Marchant S, Brailsford SR, Twomey AC, Roberts GJ, Beighton D. The predominant microflora of nursing caries lesions. *Caries Res* 2001;35:397. [PubMed: 11799279]
12. Sirinian G, Shimizu T, Sugar C, Slots J, Chen C. Periodontopathic bacteria in young healthy subjects of different ethnic backgrounds in Los Angeles. *J Periodontol* 2002;73:283. [PubMed: 11922257]
13. Tanaka S, Minami M, Murakami Y, Ogiwara T, Seto K, Shoji M, Hirata A, Abe S, Watanabe S, Fujisawa S. The detection of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* in tooth, tongue and buccal mucosa plaques in children, using immunoslot blot assay (IBA). *J Clin Pediatr Dent* 2006;30:251. [PubMed: 16683675]
14. Tanner AC, Milgrom PM, Kent R Jr, Mokeem SA, Page RC, Liao SI, Riedy CA, Bruss JB. Similarity of the oral microbiota of pre-school children with that of their caregivers in a population-based study. *Oral Microbiol Immunol* 2002;17:379. [PubMed: 12485330]
15. Tanner AC, Milgrom PM, Kent R Jr, Mokeem SA, Page RC, Riedy CA, Weinstein P, Bruss J. The microbiota of young children from tooth and tongue samples. *J Dent Res* 2002;81:53. [PubMed: 11824414]
16. Li Y, Wang W. Predicting caries in permanent teeth from caries in primary teeth: an eight-year cohort study. *J Dent Res* 2002;81:561. [PubMed: 12147748]
17. Albandar JM. Global risk factors and risk indicators for periodontal diseases. *Periodontol* 2000 2002;29:177. [PubMed: 12102708]
18. Beck JD, Slade G, Offenbacher S. Oral disease, cardiovascular disease and systemic inflammation. *Periodontol* 2000 2000;23:110. [PubMed: 11276757]
19. Van Houte J, Sansone C, Joshupura K, Kent R. Mutans streptococci and non-mutans streptococci acidogenic at low pH, and in vitro acidogenic potential of dental plaque in two different areas of the human dentition. *J Dent Res* 1991;70:1503. [PubMed: 1774381]
20. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. *J Dent Educ* 2001;65:1028. [PubMed: 11699974]
21. Caufield PW. Dental caries--a transmissible and infectious disease revisited: a position paper. *Pediatr Dent* 1997;19:491. [PubMed: 9442545]
22. Igarashi T, Yamamoto A, Goto N. PCR for detection and identification of *Streptococcus sobrinus*. *J Med Microbiol* 2000;49:1069. [PubMed: 11129717]
23. Li Y, Caufield PW. The fidelity of initial acquisition of mutans streptococci by infants from their mothers. *J Dent Res* 1995;74:681. [PubMed: 7722065]
24. Socransky SS, Haffajee AD, Smith C, Martin L, Haffajee JA, Uzel NG, Goodson JM. Use of checkerboard DNA-DNA hybridization to study complex microbial ecosystems. *Oral Microbiol Immunol* 2004;19:352. [PubMed: 15491460]
25. Kaste LM, Drury TF, Horowitz AM, Beltran E. An evaluation of NHANES III estimates of early childhood caries. *J Public Health Dent* 1999;59:198. [PubMed: 10649592]

26. Linke HA, Kuyinu EO, Ogundare B, Imam MM, Khan SH, Olawoye OO, LeGeros RZ. Microbiological composition of whole saliva and caries experience in minority populations. *Dent Clin North Am* 2003;47:67. [PubMed: 12519006]
27. Kanasi E, Johansson I, Lu SC, Kressin NR, Nunn ME, Kent RL Jr, Tanner ACR. Oral Microbiota of Pre-School Children in Pediatrician's Offices. *J Dent Res*. 2009 In press.

Figure 1a.

Figure 1b.

Figure 1. Extent of caries (dft+DFT), child age with ethnicity and immigrant status

Figure 1a. Hispanic and non-Hispanic children. There was a slight increase in caries extent with age, which was not different between Hispanic (black squares) and non-Hispanic (white circles) children by logistic regression.

Figure 1b. US born (Canadian) and immigrant (non US born) children. There was an increase in caries extent with age in immigrant children (black squares), but not the US born children (white circles). The difference was significant $p=0.047$ by logistic regression. More of the older children sampled were Hispanic and immigrant, whereas most of the younger children were US born and Hispanic (combining data from 1a and 1b).

Figure 2.

Bacterial species detected and sampling site. Species detection frequency at 10^5 cell-equivalents in matched interproximal (molar), occlusal, and tongue surface samples ($n=54$ children). *Streptococcus* species, including *S. mutans*, were the most prevalent species, followed by *Capnocytophaga* and *Selenomonas* species. Species were detected more frequently from tooth plaque samples than the tongue surface, but no comparisons were significant after adjustment for multiple comparisons.

Figure 3.

Bacterial species detected and child ethnicity. Species detection frequency at 10^5 cell-equivalents in Hispanic and non-Hispanic children. There was no difference in species detected in the molar interproximal samples between Hispanic and non-Hispanic children after adjustment for multiple comparisons.

Figure 4.

Mean species levels detected and caries extent. There were increasing levels of *S. mutans*, *S. sobrinus* and *S. salivarius* with greater extent of caries (current and prior), although there were no significant differences after adjustment for multiple comparisons. Error bars represent standard error of the mean (SEM).