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MATERNAL INFLUENCES ON THE DEVELOPMENT OF INFANT ORAL BIOFILM

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A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in Dentistry at Virginia Commonwealth University.

By

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Acknowledgement

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Abstract

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By James Joseph Dibelka, D.D.S.

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Purpose: The purpose was to examine the maternal influences on the development of infant oral biofim and dominant bacterial strains of at risk populations.

Methods: The study used a cross-sectional design to examine factors influencing biofilm colonization and the identification of bacterial strains transmitted from mother to child. Participants were enrolled in Children's Health Involving Parents of Greater Richmond (CHIP). Plaque and saliva samples were collected from mothers and their children ages 6-36 months. The colonized oral bacteria strains of the mother infant dyads were then compared. Oral bacterial strain identification was completed using the HOMIM Forsythe microbe identification array. Examination for concordant strains was done using the statistical boot strap shuffle in Excel.

Results: Forty-one CHIP families were involved in the pilot study. Participants were predominantly non-white , less than high school education 46.3%, and their average age was 29.1 years. Mothers had a caries prevalence of 87.8% and the infant's caries rate was 26.7%. To date n=14 pairs of the n=41 samples have been processed and analyzed using the HOMIM

microarray. Twelve paired samples were not processed due to non-detectable levels of bacterial DNA. Fifteen samples are currently being processed by HOMIM Forsyth.   Predominate species transferred from mother to child include S. Oralis, S. parasanguinous, S. mitis, Slakia, and S. anginosis.   425 unique strains of bacteria were analyzed on the array with a maternal concurrence rate of 33%.

Conclusion: When comparing total bacterial populations in the oral environment a concurrence of transmission from mother to child was 33%. Higher rates of vertical transmission were observed in S. Oralis, S. sanguinous, and Slakia.

INTRODUCTION

Dental caries is the single most common childhood disease with early childhood caries (ECC) disproportionately affecting minority and or low socioeconomic backgrounds[1]. Caries risk models point to definitive socio-demographic factors. These factors include income, race, birth weight, and behavior all of which influence microbial species colonization.[2] We hypothesize that maternal conditions influence the proliferation of aggressive strains of cariogenic bacteria and the subsequent establishment of a biofilm which promotes increased susceptibility to early childhood caries.[3]

Bacterial Transmission

The conceptual model of risk factors must demonstrate an understanding of the multifactorial nature of the caries process and the translational nature of the gene behavior environment on the oral health disparities in mothers and their children. An infant's mouth must be colonized by specific bacteria to be at risk for decay.[4] Much controversy exists as to when this window of infectivity exists. Studies by Li et al, [5] conclude that those children infected early and with high levels of S. mutans have significant tooth decay.[6] Conditions influencing the proliferation of these bacteria include time of inoculation, types of organisms, titer and the virulence of these specific microoraganisms.[7] Genetic profiling of oral biofilm from children

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with ECC found differences in microbial diversity and complexity when compared to a caries free dentition.[8]

HOMIM

This study will examine various pathogenic microbes (including S. mutans) in mother/infant dyads using the HOMIM-Forsyth Institute assay. The presence of virulent species will help explain from a molecular level the variance in the presentation of early childhood caries.[9] The Human Microbe Identification Microarray (HOMIM-Forsyth Institute) provides a method for rapid identification of 425 microbial species in a single hybridization.[10]

Children's Health Involving Parents(CHIP)

The CHIP health care model provides mentoring, education and behavioral health services to families in their home. CHIP providers work with parents to ensure that their children are healthy and ready for school. A typical CHIP family consists of a single female head of household, with a least two children who has not finished high school and is living in an urban environment plagued by drugs and crime.[11] Eighty percent of the families live in public housing or section VIII subsidized housing. Transmission of oral bacterial strains occur from caregiver to infant during a period of infectivity 6 months to 36 months.[12] Vertical transmission involves the movement of oral bacteria from mother to child.[13] Certain perinatal conditions may contribute to the development and establishment of a micro flora that has a greater virulence. S. mutans has been considered the primary bacteria responsible for the initiation of dental caries. Horizontal transmission however involves transmission of bacteria from non-maternal sources such as playmates or day care providers.[14]

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Study Objective

The goal of this study was to ascertain the transmission of bacteria from mother to child, then attempt to break the chain of infectivity using the CHIP format of health care delivery. This initial pilot study will be utilized as a tool for measuring the presence of bacteria and verifying the transmission of virulent bacteria to the child. Using the high-throughput technology, samples from mother infant dyads will be analyzed to determine genotypical variances among the oral microbiota.[15]

No study to date has compared the definitive DNA sequences using microarrays of the entire microbiota of the human oral cavity. The HOMIM array has the capability to definitively identify 425 unique strains of bacteria in a single hybridization.

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METHODS AND MATERIALS

Primary data collection was completed at the headquarters for CHIP of Greater Richmond. Three separate dates were offered to the mom's for participation in our study. To provide a wide dissemination to the housing localities and emphasis was utilized to incorporate the non-English speaking families of Southside Richmond. As an incentive to participate in our study the families were given a Walmart gift card and the opportunity for dental care at no cost at the VCU Pediatric Dental Clinic. As a study incentive the mothers were offered no cost dental care to reduce bacterial titers, and subsequently reduce the transmission of virulent bacteria to their children.

Biofilm was collected from a minimal pellicle of dental plaque from the facial and lingual aspects of a randomly selected primary tooth using sterile cotton swabs in the infant.[16] Plaque from mothers was collected from facial surfaces of molars using a curette, areas of frank decay were avoided. Maintaining gingival integrity was of utmost concern as to not induce a

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bacteremia or later contaminate the saliva sample. The samples were frozen immediately in dry ice and prepared using the recommended DNA extraction protocol. Sample media consisted of 1.0 ml of normal saline placed in centrifuge tubes and MoBio collection tubes. MoBio tubes contained normal saline and small inert beads.[17]

Saliva was collected from mothers in a centrifuge tube. Chewing rope wax stimulated salivary flow, sample size was approximately 5 ml. Collecting saliva as a group proved problematic, many moms became nauseous at the site of other saliva. Salivary samples where immediately frozen.

Processing of plaque and saliva was dependent on contents of the sample. Adult plaque specimens contained "chunky" amounts of plaque and debris. The MoBio DNA extraction system was utilized for adult samples. The MoBio system involved a filtering component to remove excessive debris. Then the bacterial cell was disrupted with a four-step process. Initial disruption was the result of cell breakup from the inert pellets, additional mechanical lyses was achieved via shaking of sample in the inert pellet container and adding a proprietary mixture for chemical disruption. DNA was fragmented using a recombinant lysozyme. Saliva and child plaque samples were processed via the EpiCentre MasturePure DNA Extraction Kit.[18] This technique primarily involves chemical disruption and DNA fragmentation using recombinant lysozyme. Processing microarrays required adequate DNA concentrations, the sample are then desiccated and shipped as a dried pellet to HOMIM Forsyth for development of dendogram.

This research study was approved by expedited review according to 45 CFR 46.110 Categories 3 and &7. This research involves children and is approved under 45 CFR 46.404.

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Statistical Analysis

The Fishers Exact Test SAS 9.2 was utilized to compare small sample sizes in this pilot study[19]. The Boot Strap Statistical Shuffle in Excel compared the transmission of bacteria from mother to child[20]. A statistical t test, SAS 9.2 was used to compare the caries rate in mothers to caries activity in their child[21] [22]

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RESULTS

A total of 41 mother infant dyads (n=41) participated in our study. To date n=14 of the n=41 paired samples have been processed, 12 samples were not available for processing due to non-detectable levels of bacterial DNA. 15 samples are currently undergoing microarray analysis at HOMIM Forsyth.   The health history of moms was accessed via CHIP's database. Approximately fifty percent of the data provided gestational term and birth weight. Since the moms were being seen in our clinic the missing data was obtained by questioning the parent. Literature has demonstrated an accurate recall of birth weight and gestation suitable for use in clinical and epidemiological studies.[23] [24]

Demographics

The average age of participants was 29 years with a range of 17-48 years. A descriptive analysis of the mother/infant dyads race reveals the population to be 44% black, 49% Hispanic, and 7% caucasion, with an infant male: female ratio close to 1:1. The families were all residents of the City of Richmond, VA. In analyzing the parent's education, 46% of the mothers did not have a High School Diploma, 26% had a High School Diploma or equivalent, and 28% had some college. When comparing caries rate and education levels, children of parents with education

less than High School had a significantly higher rate of caries, p=.031. These results are summarized in table 3

Nearly all moms experienced decay, however the caries rate in their children was significantly lower. There was a marginal but not statistically significant correlation between caries activity in parents and their children, p=0.058. These results are summarized in figure 1.

HOMIM

Microarray results presented as a dendogram representing the relative intensities of bacterial concentrations in each sample. Intensities were classified using a scale of 0-5 distinguished by the brightness of green illumination. 425 unique genetic markers were available for hybridization, with a positive microbe identification of 194 bacteria. Concurrence of bacterial similarities was established using the Boot Strap Shuffle Statistical Technique. A single positive hybridization for specified bacteria was randomly matched to each child in the sample pool. The result of the statistical shuffle yielded a concurrence of bacteria present in the oral cavity in mother infant dyads of 34% with a p=0.31. The concurrence demonstrates an attempt to randomly match a mother to child using positive hybridization as the sole determinant.

Perinatal data was collected from linking to the CHIP database. The criteria evaluated in this study include birth weight and gestation time. Appoximately 50 % of the perinatal data was available through the CHIP database. Missing data was supplemented by asking mothers birth weight. One child presented with a history of very low birth weight, 2240 g. The bacterial profile did not indicate any trends in colonization substantially divergent from normal birth weight individuals. The only difference in colonization was noted in low birth weight children by the presence of Solbacterium moorei that was vertically transmitted from the mother.

DISCUSSION

The population of mother/infant dyads enrolled in this study consisted primarily of Hispanic and African American individuals from the City of Richmond, VA. Most of the caries activity in this group was found in the Hispanic population. In addition children of mothers who have not completed High School demonstrated the highest caries activity.

The HOMIM microarray presented enormous amounts of data for interpretation. The limited scope of the pilot study permits only a partial analysis the data. Previous studies have compared one or two species of the Viridans group with respect to vertical transmission from mother to child.[25] [26] [27] These studies would establish the presence of bacteria usually S. mutans in the mother and then observe whether this stain was colonizing within the child's microbiota. Early identification by Caufield et al[28] involved colonization of bacteria on restrictive agar plates of S. mutans or later by the Mitchell group by 16s primers of key components of S. mutans DNA. [29] No study to date has compared the definitive DNA sequences using microarrays of the entire microbiota of the human oral cavity. The HOMIM array has the capability to definitively identify 425 unique strains of bacteria in a single hybridization.

The process of managing 194 positive hybridizations in the oral microbiota and comparing these bacteria in the mother infant pairs required unique statistical techniques. We utilized the Statistical Boot Strap Shuffle in Excel to match bacteria present in a mothers unique flora to that of all the microbiotas present in the pool of children hybridizations. In other words, we matched the mother to each child randomly seeking a concurrence in bacteria present in both floras. The results of matching 14 mothers randomly to 14 children yielded a match in bacteria colonies correctly in 33% of the dyads. The transmission or concurrence rate of all bacteria compares similarly to the reported transmission rate of Streptococcus mutans, which ranges from 37 to 71 percent [30] [31] [32]. Past studies did not conclusively identify S. mutans as the only colony present in the flora. Contamination was likely, therefore the transmission rates previously identified as S. mutans likely contained numerous species.

The lack of S. mutans as a dominant species in the majority of mother/infant dyads in both caries free and carious individuals suggests the likely activity of other bacteria in development of decay. This data is confirmed by another microarray study by Tanner et al.[33] Vertical transmission of S. mutans between this group of mother infant dyads was not observed. Previous studies by the Alabama group suggest a transmission rate of 41% for S. mutans, however collection of bacteria samples in this study involved retrieving plaque from carious lesions. S. mutans colonization identified by hybridization in a microarray has demonstrated this occurs only late in the caries process and within the lesion. HOMIN Forsyth technology has shown that S. mutans is only present within a lesion or late in the demineralization process and rarely in supragingival plaque. Our arrays have also demonstrated in a single case where S. mutans was present in large amounts other Viridians stains have limited abundance in the microbiotic niche.

This study did observe vertical transmission of other Viridans groups between a mother and her child. S. anginosus, S sanguis, S. parasanguis and S. mitis all had significant similarites in bacterial colonization. S. Mutans was a dominant strain in the microbiota of one child. A total of four mothers demonstrated the presence of S. Mutants. No evidence of vertical transmission occurred among the S. mutants strains p=0.082. This is summarized in table 4. It was observed in the one child where S. mutants was the dominant strain, with relative intensities of four and five, limited colonization of other Streptococcus strains was noted. In addition a total absence of colonization occurred with Slakia exiguia, S. anginosis, S. sanguinis, S. parasanguinis, S. oralis, and S. mitis. It appears the limited abundance of these species would indicate the microbiologic niche of the flora was occupied by S. mutans.

Other members of the Viridans group of commensal oral bacteria that were evaluated in the microarray include S. anginous, S. sanguis, S. oralis and S. mitis. A very high correlation exists between the colonization of these bacteria in the mother infant dyads. Prevalence rates between mother and child range from a high of 100 percent for S. oralis to 63 percent for S. mitis bv2.

Two additional groups of bacteria common to mother and child were the Leptotrichia buccalis and Slakia exigue strains. Each of these strains demonstrated a prevalence rate of 93 percent. These results are summarized in table 5.

Limitations

To date HOMIM has processed 14 dyad sample sets n'=14. Many challenges developed in processing our plaque samples. MoBio was developed to manage high concentration samples, therefore adult plaque easily provided adequate DNA titers. Attempting to extract DNA using the Mobio technique from child plaque and saliva could not produce sufficient genomic DNA

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concentrations. The EpiCentre Masture Pure DNA extraction system was developed to manage low concentrations of sample material. We initially were concerned about the compatibility of storing samples in Mobio vs. Epicentre, the manufacturer of Mobio was contacted and disclosed the content of the tubes contained normal saline, therefore no procedural discrepancy would be expected.

CONCLUSIONS

The aim of this study was to evaluate maternal influences on the development of infant oral biofilm. Mother/infant dyads were selected from a lower socioeconomic population participating in Children's Health Involving Parents of Greater Richmond. High throughput technology was used to definitively identify 425 unique bacterial strains colonizing the oral flora.

- When comparing the transmission of the 194 colonizing bacteria profiles present in the mother and child a concurrence rate of 33% was observed.

- Streptococcus Mutans was not the dominant species within the mother/infant microbiotas.

- Streptococcus Mutans did not demonstrate vertical transmission tendencies.

- S. anginosus, S. sanguis, S. oralis, S. parasanguis, and S. mitis did demonstrate a high degree of prevalence between mother and child.

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Table 1: Demographic background of participants

Race/Education	N=41	Percent
African American	18	44
Hispanic	20	49
White	3	7
Less than High School	19	46
High School/GED	11	26
Some College	13	28

Table 2: DMFT/dmft scores for mother infant dyads.

	N	DMFT avg	Median	St. DEV	MAX
Mothers	41	10.4	12	6.9	27
Child	45	1.3	0	3.4	20

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Table 3: Child's incidence of decay compared to mom's education, Fishers Exact Test SAS 9.2, p=0.031.

Education	dmft=0	dmft=1-4	dmft>4	ndmft=0	ndmft=1-4	ndmft>4
<HS	2	3	2	10	5	2
HS	4	0	0	12	1	0
Some coll	3	0	0	9	0	3

Figure 4: Prevalence of S. mutans in mother/infant dyad

S. Mutans Prevalence	S. Mutans (+)	S. Mutans (-)
Mother caries (yes)	3	10
Mother caries (no)	0	1
Child caries (yes)	1	4
Child caries (no)	0	10

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Table 5:   Prevalence of transmitted bacteria in mother/infant dyads.

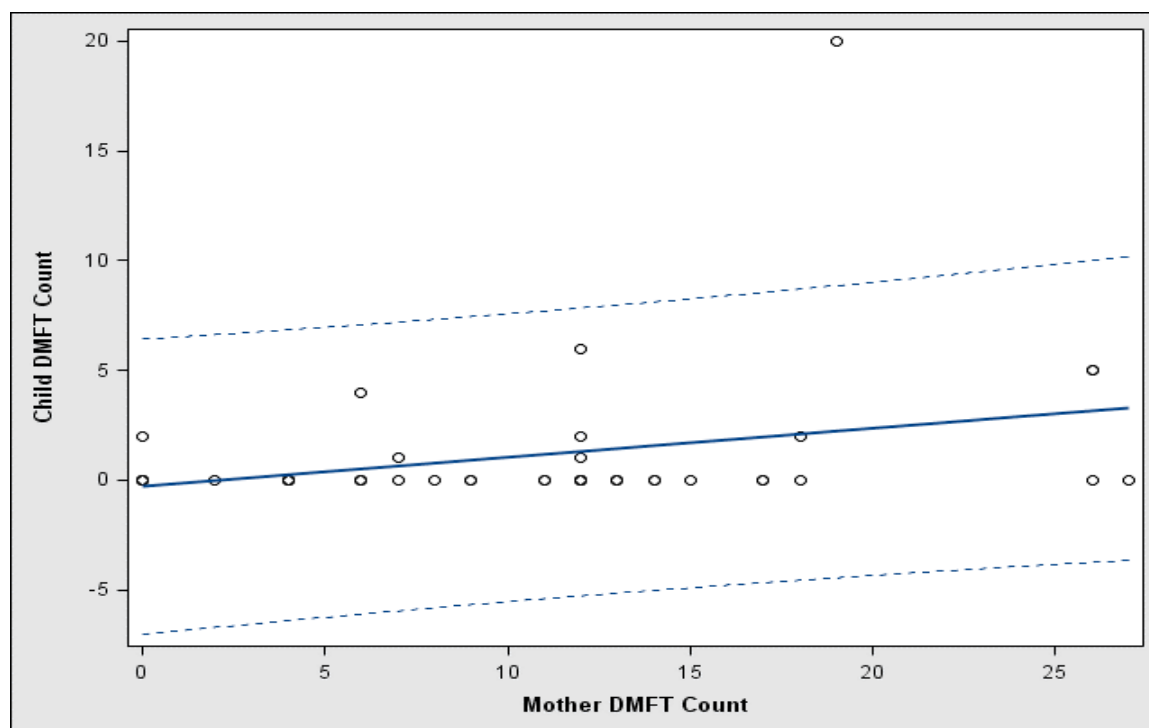|	Trans %	Moms +	Kids +	mom + kid -	mom- kid +	mom- kid-
S. anginosus and intermediates	86	14	12	2	0	0
S. sanguis	85	13	11	3	1	0
S. oralis	100	14	14	0	0	0
S. parasanguis	86	14	12	2	0	0
Slakia exiguia	93	13	13	1	1	0
Leptotrichia buccalis, goodfellow	93	13	14	0	1	0
S. mitis bv2	61.5	13	9	4	1	0

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Figure 1: No correlation between mother and infant caries activity, Fishers Exact Test SAS 9.2

p= 0.058.

Literature Cited

Literature Cited

1. Kaste L, Sreenivasan D, Koerber A, Punwani I, Fedvi S. Pediatric Oral Health Knowledge of African American and Hispanic of Mexican Origin Expectant Mothers. Pediatric Dentistry 2007; 29:287-292.
2. Ripa, L, Nursing Caries: A Comprehensive Review, Pediatric Dentistry, 1988. 10(4): p. 268-282.
3. American Academy of Pediatric Dentistry: Clinical Guidance on infant oral health care Pediatric Dentistry. 2004-05, p. 67-69.
4. Alaluusa, S. and O. Renkonen, Streptococcus mutans establishment and dental caries experience in children 2 to 4 years old. Scandinavian Journal of Dental Research, 1983. 91: p. 453-457.
5. Twetman, S., Stahl, B, and T. Nederfors, Use of the strep mutans test in assessment of caries risk in a group of preschool children. International Journal of Pediatric Dentistry, 1994. 4: p. 245-250.
6. Li Y, Ge Y, Saxena D, Caufield PW, Genetic Profiling of the Oral Microbiota Associated with Severe Early Childhood Caries. Journal of Clinical Microbiology, 2007. 45(1): p. 81-87.
7. Kanasi E, Douglas C W, Okuji DM, Merabi S, Boches SK, Dewhirst FE, Paster BJ, Tanner AC. Microarray Analysis of Microbiota of Young Children. Journal of Dental Research. 87 (#3418.): 2008. Http:// mim.forsyth.org/index.html.
8. Fadavi S., Punwanj, I and Dharmapuri, Prevalence of Dental Caries in Prematurely-born Children, Journal of Pediatric Dentistry, 1993. 17(3): p. 163-65.
9. Warren JJ, Weber-Gasparoni K, Marshell TA, Drake DR, Dehkordi-Vakil, Kilker JL, Dawson DV. Factors Associated with Dental Caries Experienced in One Year Old Children. Journal of Public Health Dentistry, 2008. 68(2): p. 70-75
10. Background Human Microbe Identification Microarray, www.mim.forstyh.org/homim
11. Children's Health Involving Parents, One Family at a Time,www.chip.org
12. Aaltonen AS, Tennuovo J., Association between Mother Infant Salivary Contacts and Caries Resistance in Children, Pediatric Dentistry, 1994; 16:110-6.
13. Law V, Seow WK, Townsend G, Factors Influencing Oral Colonization of Mutans Streptococci in Young Children, Aust Dental Journal, 2007; 52:93-100.
14. Tedjosasongko U, Kozai K, Initial Acquisition and Transmission of Mutans Streptococci in Children at Day Nursery, Journal of Dentistry Child 2002:69:284-8.
15. Human Microbe Identification Mircroarray,www.HOMIM.org
16. Fabiana PM, Gonzalez-Ittig RE, Initial Acquisition and Genetic Identity of Streptoccous mutans of Mother Child Pairs, Pediatric Dentistry, V32/ NO 3, May/June 2010, 205-11.
17. PowerSoil DNA isolation kit, www.mobio.com/soil-DNA.
18. MasterPure complete DNA and RNA Purification Kit, www.epibio.com
19. www.ats.ucla.edu/stat/sas/whatstat/whatstat/htm
20. www.wabash.edu/econometrics/EconometricsBook/Basic%20Tools/ ExcelAddIns/bootstrap
21. Hill, T. & Lewicki, P. (2007). STATISTICS Methods and Applications. StatSoft, Tulsa, OK

22. Dawson B, Trapp R. Basic and Clinical Biostatistics, McGraw Hill Medical, 4th ed (2APR2004)

23. Seidman DS, Slater PE, Ever-HadaniRG, Accuracy of Mother's Recall of Birth Weight and Gestational Age, BJOG: An International Journal of Obstetrics and Gynaecology, Vol 94, 731-35, AUG 87.

24. Allen DS, Ellison GT, Determinants of the Availability and Accuracy of Self Reported Birth Weight in Middle Aged and Elderly Women, American Journal of Epidemiology, Vol 155,4 379-384.

25. Berkowitz RJ, Acquisition and Transmission of Mutans Streptococci. J California Dental Association 2003:31:135-8

26. Caufield, PW, Cutter GR, Dasanayake AP. Initial Acquisition of Mutans Streptoccoi by Infants: Evidence for a Discrete Window of Infectivity. Journal Dental Research, 1993;72:37-45.

27. Douglass JM, Li Y, Tinanoff N. Association of Mutans Streptococci between caregivers and their children. Pediatric Dentistry 2008;30:375-87.

28. Caufield PW, Wannemuehler Y, Hansen HB. Familial Clustering of the Streptococcus Mustans Cryptic Plasmid Strain in a Dental Clinic Population. Infective Immunity 1982;38: 785-7.

29. Mitchell SC, Ruby JD, Moser S et al, Maternal Transmission of Mutans Streptococci in Severe-Early Childhood Caries, Pediatric Dentistry, Vol 31 / No 3 May/JUN 2009.

30. Li y, Caufield PW. The Fidelity of Initial Acquisition of Mutans Streptococci by Infants from their Mothers. Journal of Dental Research 1995:74:681-5.

31. Emanuelsson IM, Wang XM. Demonstration of identical strains of mutans streptococci within Chinese families. Eur Journal of Oral Science, 1998;106:788-94.

32. Tedjosasongko U, Kozai K. Initial aquistition and transmission of mutans streptococci in children at day nursery. Journal of Dent Child 2002;69:284-88.

33. Tanner et al. Microbiota of severe early childhood caries by microarray and PCR. J Dent Res 2011; 90:240.

VITA

James Joseph Dibelka Jr. was born January 27th, 1956 in San Diego, CA. He graduated from the University of California, Davis in 1978, and received his Bachelor of Science in Biochemistry. Dr. Dibelka then received his Doctor of Dental Surgery from Virginia Commonwealth University in 1983. He then practiced general dentistry from 1983 to 2003 in both Virginia and California. During this time period he was recalled to active duty to support Operation Iraqi Freedom and was assigned to MCB Camp Pendleton. Upon completion of his residency in Pediatric Dentistry CAPT Dibelka will transition to the 3rd Dental Battalion, Okinawa, Japan.