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Multi-generational Transmission of Mutans Streptococci in Early Childhood Caries

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Multi-generational colonization by mutans streptococci in early childhood caries

by

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THESIS

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ABSTRACT

Multi-generational Transmission of Mutans Streptococci in Early Childhood Caries. Rahman JE*, Featherstone JDB, Zhan L, Hoover CI (University of California San Francisco, School of Dentistry, San Francisco, CA)

Purpose: To determine the extent of multi-generational (grandmother-mother, mother-child, and grandmother-grandchild transmission of mutans streptococci (MS) in early childhood caries (ECC) families where grandmothers provide significant childcare (≥20 hours/week).

Methods: Ten grandmother-mother-child triads (30 participants) were recruited using a questionnaire. All children (aged 3-6 years) had at least one active carious lesion. DMFS/dmfs scores were determined using WHO criteria, and saliva samples were obtained. Saliva samples were cultured on MSSB agar to enumerate and isolate MS. Ten MS colonies were randomly selected and propagated for arbitrarily primed-polymerase chain reaction (AP-PCR) typing using two primers (OPA-5 and OPA-13).

Results: MS were isolated from all subjects except one grandmother, who had no MS detected. There were no significant differences in log_{10} MS CFU/mL of saliva (4.4±1.6, 4.6±1.0, 4.8±1.0, for children, mothers, and grandmothers, respectively). There were significant differences (P≤.05) in DMFS/dmfs scores between grandmother-mother, mother-child, and grandmother-child (DMFS/dmfs Mean±SD, grandmother: 55.3±21.8, mother: 15.4±8.3, child: 33.4±16.3). Fermentation tests were used to differentiate between S. mutans and S. sobrinus. AP-PCR results demonstrated a 30% transmission rate from mothers to children, and a 44% transmission rate from grandmothers to children. Of all genotypes found in children, 14 out of 22 (64%) genotypes were from neither grandmother nor mother, and therefore from an unknown source.

Conclusion: The results indicate transmission of MS between grandmother-mother, mother-child, and grandmother-grandchild in ECC families where the grandmother provides significant childcare. This provides evidence to support more broadly based family-oriented strategies to reduce transmission of MS and the incidence of ECC.

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1. **STUDY AIM/PURPOSE**

The purpose of this study is to identify specific strains of mutans streptococci (MS), namely *S. mutans* and *S. sobrinus*, which are related to the vertical transmission of MS from mother and grandparent to child. The hypothesis to be tested is that the MS is transmitted vertically from the mother, as well as the grandmother who is providing care for the child on a regular basis.

2. **LITERATURE REVIEW**

Early childhood caries is defined as “the presence of 1 or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces” in any primary tooth in a 71-month or younger child. In children younger than 3 years of age, any sign of tooth smooth surface caries is indicative of severe early childhood caries (S-ECC). Most experts agree that multiple factors influence the initiation and progression of the disease. For dental caries to occur, there must be three requirements: 1) a host (the tooth in the oral environment), 2) a substrate (a fermentable carbohydrate), and 3) cariogenic bacteria. The substrate serves as a nutrient source for the bacteria, after which the bacteria produce acids as a by-product of metabolism, which causes demineralization of the tooth surface and, subsequently, caries.

ECC is an infectious and transmissible disease, and MS (including *Streptococcus mutans* and *Streptococcus sobrinus*) are the most likely causative agents. The oral environment has evolved in such a way that the bacteria which inhabit the human mouth appear to have a mutualistic relationship with each other. These organisms live together through the exploitation of very specific ecological niches. For example, lactobacilli are
known to favor the dorsum of the tongue, while *Streptococcus mutans* requires a solid, non-shedding surface for colonization. In addition, certain bacteria rely on other “pioneer” bacterial species to survive in the human mouth. For example, the sanguinis streptococci group produces nicotinamide adenine dinucleotide (NAD), which is then consumed by *Haemophilus parainfluenzae* (Liljemark and Bloomquist 1996).

*Streptococcus mutans* has been implicated as being the initiator of dental caries due to its multiple virulence factors, the most important being that *S. mutans* is aciduric (able to live in acidic environments) and acidophilic (prefers acidic environments). Unlike many of the other oral bacteria, *S. mutans* has the ability to thrive under acidic conditions. This acidic environment provides a favorable environment for its metabolic processes, whereas the low pH causes the metabolism of other microorganisms to be slowed down, allowing *S. mutans* to be the dominant bacteria.

Early acquisition of MS in the oral cavity has been documented as a major risk factor for early childhood caries (Berkowitz 2003) as well as future caries experience (Alaluusua and Renkonen 1983; Kohler, Andreen *et al.* 1988; Fujiwara, Sasada *et al.* 1991; Roeters, van der Hoeven *et al.* 1995; Grindfjord, Dahllof *et al.* 1996; Wan, Seow *et al.* 2003). An important route of early acquisition of MS in humans is vertical transmission from caregiver to child. The major reservoir from which infants acquire MS is their mothers. Mothers who have higher *S. mutans* levels, poor oral hygiene, frequent sugar exposures, and child-rearing habits that facilitate saliva transfer, such as routinely sharing food and utensils, generally infect their children at an earlier age (Wan, Seow *et al.* 2003). A study by Berkowitz *et al.* (1975) reported that, when mothers harbored greater than $10^5$ colony forming units (CFU) of MS per mL of saliva, the frequency of
infecting their infant was 58%. When mothers harbored $10^3$ CFU of MS per mL of saliva or less, however, the frequency of infant infection was 9 times less (6%). This study demonstrates that mothers who have high numbers of MS are at significantly increased risk for infecting their infants early in life.

Early studies utilizing bacteriocin typing demonstrated that MS isolated from mothers and their infants showed identical bacteriocin typing patterns (Berkowitz and Jordan 1975; Davey and Rogers 1984; Berkowitz and Jones 1985). More recent evidence for this concept emerged with newly advanced technology that utilized chromosomal DNA patterns or plasmid profiles (Caufield, Childers et al. 1985; Kulkarni, Chan et al. 1989). These studies provide further convincing evidence to confirm the theory of vertical transmission.

A large scientific effort has been made to reduce the vertical transmission from mother to child by decreasing the numbers of MS. Dasanayake et al. demonstrated that short-term application of iodine-NaF varnish in mothers with high MS counts did lower MS in the mothers’ saliva, but it did not influence the incidence and timing of the acquisition of MS or caries experience in their children (Dasanayake, Caufield et al. 1993). Other studies investigating antimicrobial techniques show a reduction in the numbers of MS, but generally no reduction in MS colonization or caries development in their children. One study utilizing a chlorhexidine varnish in mothers with high MS counts showed that the frequency of MS colonization of their children was not significantly different from the control group (Gripp and Schlagenhauf 2002); this was confirmed in another study (Dasanayake, Wiener et al. 2002). Topical applications of kanamycin and vancomycin have also been studied (De Paola, Jordan et al. 1974;
Loesche, Bradbury et al. 1977). While these antimicrobials did show decreases in MS counts, reduction in caries primarily occurred only on smooth surface areas of the tooth. Scientists have suggested that these antimicrobial therapies may have limited effectiveness due to their inability to penetrate into pit and fissures, margins of restorations, and proximal surfaces of teeth.

Recently, there have been a number of studies that have demonstrated the anticariogenic effect of xylitol. Xylitol is a five carbon sugar substitute with sweetness equal to that of table sugar (sucrose), but with 40% fewer calories. Researchers have shown that xylitol significantly reduces the mother-child transmission of *S. mutans* as compared to other preventative measures such as chlorhexidine and fluoride (Soderling, Isokangas et al. 2001; Thorild, Lindau et al. 2006). Commercially available products containing xylitol include gums, mints, energy bars, toothpaste, mouthrinses, and dental wipes. Unfortunately, a majority of these products do not contain enough xylitol to have an anti-cariogenic effect. Additionally, most products do not contain the specific amount of xylitol on the packaging, which makes it difficult for consumers to make informed decisions about which products to purchase for the prevention of caries (Ly, Milgrom et al. 2006).

While the concept of vertical transmission from mother to child has been widely studied, transmission of MS from grandparent to child requires further research. Since the 1970s, trends in grandparent caring for grandchildren have become more prevalent. The number of children in such households grew from 2.2 million (3.2%) in 1970 to 3.9 million (5.5%) in 1997, with particularly rapid growth since 1980 (Lugaila 1998). In 1997, grandparents were the leading child-care providers for preschoolers who were in
some type of child-care arrangement; among the nation's 19.6 million preschoolers, grandparents cared for 21% of these children (Johnson 2005). Since 1990, there has been a 30% increase in the number of children (half of whom are under the age of 6) living in households maintained by grandparents (Census 2000; Fuller-Thomson and Minkler 2001). In 2000, 5.7 million grandparents lived with their grandchildren (Bryson 2001; Census 2001), and approximately 2.4 million grandparents were raising their grandchildren.

Many families make arrangements with grandparents to watch the grandchildren while the parents are at work. Reasons for this arrangement include avoiding the rising cost of day care and the desire of having the children with someone they know and trust. Grandparents who care for their grandchild, with the middle generation present, provide an affordable daycare option for their working children (Goodman and Silverstein 2002); in addition, the middle generation and children may live with the grandparents for financial reasons (Jendrek 1994). In addition, grandparents can serve as role models for the grandchildren. Improved school performance, less reliance on welfare, more autonomy in decision making, and fewer deviant behaviors have been observed in families where grandparents provide childcare (Wilson 1986). Most importantly, grandparents are able to provide love, security, encouragement, and structure for their grandchildren.

The elderly population is a major reservoir for MS. In close family situations, grandparents may be the initial route of transmission of cariogenic bacteria to young children. It is known that dentures can serve as colonization sites for MS (Jordan and Keyes 1966; Carlsson, Soderholm et al. 1969). Carlsson et al. (1969) demonstrated the
presence of streptococcal species (i.e. S. sanguinis and S. mutans), in edentulous individuals who wore dentures. It seems that dentures, as well as teeth, provide an environment which is suitable for the colonization of MS. In addition, MS disappeared from the mouths of edentulous individuals when they stopped wearing their dentures, and reappeared once the individuals resumed wearing them. Fitzgerald et al. (1983) also showed that the distribution of different species and types of MS in the elderly population was similar to the distribution seen in younger age groups. Furthermore, the prevalence and virulence of MS appeared to be very similar in both naturally dentate individuals and full-denture wearers (Fitzgerald, Fitzgerald et al. 1983). The elderly population in general, low-income groups in particular, tend to show an increased preference for sweets and high-carbohydrate diets, which favor the colonization and increase of MS (Nizel 1972). The older population is also retaining teeth for a longer period of time (Burt 1999), which when included to the cumulative effects of gingival recession, various drugs that cause reduced salivary function, and poor oral hygiene and dietary habits, increases their susceptibility to caries. It is important to recognize that elderly individuals, dentate or edentulous denture wearers, represent a significant reservoir for MS. These individuals who are in close familial contact with infants and young children may be potential vectors in the transmission and colonization of cariogenic bacteria.

Arbitrary primed polymerase chain reaction (AP-PCR) is one of the most widely used methods in differentiating between various strains of MS. AP-PCR is a molecular biological technique used for exponentially amplifying random DNA segments of the target bacterial species with arbitrary sequenced primers (Saarela, Hannula et al. 1996). One of the main advantages of AP-PCR is that no prior knowledge of the DNA sequence
of the target bacterial species is required (Saarela, Hannula et al. 1996). In addition, AP-PCR is a relatively quick and simple technique, making it an excellent alternative to hybridization techniques. AP-PCR has good discriminative ability in genotyping MS species when compared with the standard ribotyping method (Saarela, Hannula et al. 1996).

Vertical transmission of MS from caregiver to child is an important component in the initiation and progression of dental caries. Different studies report varying frequencies of maternal vertical transmission, ranging form 24% to 71% (Alaluusua and Renkonen 1983; Li and Caufield 1995; Alaluusua, Matto et al. 1996). This variability may be due to the fact that transmission depends on several factors, including the frequency of salivary contact between mother and child, a mother’s salivary MS level, and the cultural and environmental conditions of the population studied. In addition, variability in transmission can also be associated with children’s individual susceptibilities, including the period defined as a window of infectivity (Caufield, Cutter et al. 1993), the number of erupted teeth (Caufield, Cutter et al. 1993); the emergence of molars (Caufield, Cutter et al. 1993); the presence of enamel hypoplasia; carbohydrate consumption; the action of the salivary and mucosal immune systems (Li and Caufield 1995), and immunological conditions in children (Smith, King et al. 1998). As the child gets older and broadens his or her social contacts, horizontal transmission, which is the transmission of bacteria between members of a group (eg, family members of a similar age, or students in a classroom), becomes more prominent. This, in turn, decreases the frequency of mother-child genotype matching of MS. One study by Mattos-Graner et al. (2001) demonstrated matching genotypes of MS strains in non-related children at a
Brazilian nursery, which strongly suggests horizontal transmission. This additional source of MS from daycares and nurseries can increase the number of MS genotypes present in the child’s oral flora. One study by Tan (2007) demonstrated a 40% transmission rate from mother to child, which suggests other strains of MS must have originated from elsewhere. The identification of significant sources of MS transmission is essential in the development of strategies towards the prevention of caries.

To date, no previous studies have documented transmission of MS from childcare-providing grandmothers to grandchildren and/or multi-generational transmission of MS (grandmother-mother-child). ECC tends to be more frequent in disadvantaged socioeconomic populations where grandmothers often provide significant childcare. In these situations, grandmothers may be an unrecognized reservoir for transmission of MS to children. The model from this study has the potential to develop into a screening method for detection of high caries-risk children, as well as create an additional education tool to avoid MS transmission and dental caries in children. Detailed knowledge regarding the acquisition and transmission of cariogenic bacteria may provide evidence to support the development of more broadly based family-oriented strategies to reduce the incidence of ECC.

2.1 Hypothesis

MS may be transmitted vertically in a multi-generational manner including grandmother-mother, mother-child, and grandmother-child, particularly when the grandmother is providing routine childcare (≥ 20 hours per week).

2.2 Significance
Early acquisition of MS leads to higher caries prevalence in young children and subsequently in adulthood. By preventing vertical transmission of MS strains from caregiver to the child, the risk of developing future caries in the child will be substantially reduced. Understanding transmission of MS is essential for developing effective strategies to prevent dental caries in young children.

3. MATERIALS AND METHODS

3.1 General Study Design

Vertical transmission of MS between grandmothers and mothers to children was investigated in 10 grandmother-mother-child triads. The study was approved by The Committee on Human Research of the University of California San Francisco, approval number H5954-31237, dated July 6, 2007, with 2 annual renewals.

Subjects who fulfilled the inclusion criteria were recruited for this study until 10 grandmother-mother-child triads were enrolled. Approximately 25 triads were screened to identify 10 qualifying triads. All 10 triads consented to participating in this study. The number of decayed, missing, or filled permanent surfaces (DMFS) was determined through oral examination for grandmothers and mothers, and number of decayed, missing or filled primary surfaces (dmfs) for children. Stimulated saliva from mother and grandmother, and swab saliva samples from the child were taken for determination of MS colonization levels. Ten typical MS colonies were randomly isolated from each subject with MS infection. Fermentation tests were conducted to differentiate between S. mutans and S. sobrinus strains. AP-PCR was used for the genotypic characterization of the MS strains. The strains present in the mother-child and grandmother-child pairs were
considered the transmitted strains. The strains that were present only in the mother or grandmother but not the child were considered non-transmitted strains.

3.2 Subject Selection and Recruitment

3.2.1 Initial Contact Method

UCSF pediatric dental residents, staff and faculty were briefed on participant recruitment. Posters were placed at the reception desk and throughout the pediatric dental clinic to notify patients of the study (Appendix 1). Children 3-6 years old and their accompanying parent and grandmother were approached for recruitment. All participating children were patients of record at the UCSF Pediatric Dental Clinic and were scheduled to be seen by a pediatric dental resident or pre-doctoral student.

3.2.2 Inclusion/Exclusion Criteria

Inclusion criteria:

1. Children ≥ 3 years-old and < 6 years-old with at least one active carious lesion.
2. Accompanying mother and grandmother must be able to understand the basic procedures associated with the study and the language usage of the consent form.
3. Grandmother must be a primary caregiver of the child (i.e. provides childcare ≥20 hours per week).

Exclusion criteria:

1. Children with developmental dental diseases &/or malformed dentition.
2. Children who use medications that may influence oral microbial flora
(i.e. broad spectrum antibiotic use within the last 6 months).

3. Children with significant current or past medical conditions that may affect oral health and oral microbial flora (i.e. pregnancy, diabetes, HIV, heart conditions that require antibiotic prophylaxis during dental procedures, etc.)

Grandmothers and mothers who fulfilled the inclusion/exclusion criteria and agreed to participate in the study were asked to complete a detailed questionnaire regarding age, ethnicity, feeding, and oral hygiene habits prior to final enrollment (Appendix 2).

3.2.3 Consent

Informed consent was obtained by the main investigator from the grandmothers and mothers (Appendix 3). Procedures, benefits, risks and rights of the subject in the study were discussed with the mothers. Because the children were too young to give written or verbal assent, the mothers were asked to sign a permission form to allow children to participate in the study. Subjects were provided ample time (30 minutes) to accept or decline participation in this study.

3.2.4 Dental Examination

DMFS/dmfs examinations using the World Health Organization (WHO) criteria in a regular dental setting without radiographs were conducted on the grandmother, mother, and child respectively prior to saliva collection (Appendix 4).

3.2.5 Saliva Sample Collection

Stimulated Saliva Collection from the Mother
Grandmothers and mothers were asked to chew on a piece of paraffin wax and expectorate into a test tube until 3 mL of stimulated saliva were collected.

**Swab Sample Collection from Child**

Oral swab samples were obtained from children at least one hour after a meal, at least 2 hours after tooth brushing, and prior to any dental treatment. A sterile cotton tipped applicator (CITMED Citronelle, AL.) was used to collect saliva from tooth surfaces, gingiva, tongue and oral mucosa. The swab tip was broken off and dropped into a pre-labeled 5 mL sample tube with 2 mL of phosphate-buffered-saline (PBS, pH 7.2).

Saliva samples were transported on ice to a microbiology laboratory at UCSF for MS plating and culture within 24 hours of collection.

**3.2.6 Participant Reimbursement**

Each triad received $30 (in the form of Target® gift cards; $20 to the mother and $10 to the grandmother) to compensate their time in the study upon saliva collection from the last member of the triad.

**3.2.7 Confidentiality of Records**

All subjects’ personal information, such as names and addresses, was kept in a locked cabinet. Information gathered from the participant survey such as ethnicity, oral and dental habits were collected and recorded by the main investigator at the time of recruitment. Research records were handled confidentially. All records were coded with study numbers and kept in locked files so only study investigators had access to them. No individual identities were used in any printed material or reports from this study.

**3.3 LABORATORY PROCEDURES**

**3.3.1 Mutans Streptococci Enumeration**
The test tubes containing the saliva samples from the mother and grandmother were sonicated for 20 seconds, and 0.1 mL portions were removed for microbiology assays, as described below. Swab samples were vortexed for 30 seconds and 0.1 mL portions of resulting suspension were removed for microbiology assays, as described below. Ten-fold serial dilutions ($10^{-1}$ through $10^{-5}$) of the dispersed oral samples were prepared in PBS. One-tenth mL of the sonicated or vortexed oral samples and 0.1 mL of each serial dilution were plated on Mitis Salivarius Sucrose Bacitracin agar (MSSB) to selectively culture MS. Plates were incubated at 37°C with anaerobic conditions of 95% N₂, 5% CO₂, 10% H₂ for 48 hours before enumeration of MS colonies under a dissection microscope. Results were recorded as CFU/mL of saliva or CFU/mL of suspended swab sample. After enumeration, MS colonies that exhibited different colonial morphologies and then random colonies were selected for isolation from each subject. Ten colonies were selected from each subject. Each selected colony was streaked on another MSSB plate and after incubation as above, streaked on a BHI plate for pure culture. The pure culture isolates were then stored in TSB glycerol broth at -80°C for future assays.

### 3.3.2 Fermentation Tests

Differentiation of *S. mutans* and *S. sobrinus* was based on fermentation tests of sorbitol, mannitol, melibiose and raffinose. Briefly, 0.1 mL of bacteria suspension stored at -80°C was inoculated into 2 mL of TPY broth (contains 1.5% tryptone, 0.4% polypeptone, 0.4% yeast extract, 1% glucose, 0.5% KH₂PO₄, 0.25% Na₂CO₃ and 0.2% NaCl). The broths were then incubated for 18 hours
anaerobically. Then 0.1 mL of the bacteria culture in TPY broth was inoculated into 1 mL of TPY broth with 1% sorbitol, mannitol, melibiose or raffinose but no glucose. Bromcresol purple was used as an indicator for fermentation tests. The cultures were again incubated for 48-72 hours before evaluation. A color change from purple to yellow indicates a positive fermentation test. *S. sobrinus* only ferments sorbitol and mannitol, whereas *S. mutans* can ferment all four sugars.

### 3.3.3 Genomic DNA Extraction

Genomic DNA from each pure culture MS isolated was archived on FTA Matrix Cards (Whatman BioScience, Massachusetts, USA) and portions of the FTA cards were used for AP-PCR analysis. One-tenth mL of the -80°C stored MS isolates was streaked onto MSSB, incubated as previously described, and then cultured on a BHI agar plate. A few MS colonies were collected on a sterile cotton swab (CITMED Citronelle, AL) and inoculated into 2 mL of TPY broth and incubated overnight as previously described.

A micropipette was used to place 100 µL of the MS TPY broth onto a Classic FTA Card. The card was allowed to dry overnight at room temperature (RT) and then stored at RT until needed for AP-PCR.

### 3.3.4 Arbitrary Primed Polymerase Chain Reaction

A micro punch was used to remove a 2 mm portion of the FTA Card MS sample. The punch was transferred to a 250µL PCR amplification tube. FTA Purification Reagent (200µL) was added to each tube and incubated for 5 minutes at room temperature. The reagent was removed with a micropipette. This process was repeated twice. After the third wash and removal of FTA Reagent, 200µL of
TE Buffer was added to each tube and incubated for 5 minutes at room temperature. A micropipette was used to remove the TE Buffer and this TE Buffer wash was repeated. After removal of the final TE Buffer wash, the punch was rinsed with ethanol, dried in a 56°C incubator for 10 minutes, and then used for AP-PCR.

AP-PCR with two primers, OPA-5 and OPA-13, used separately was performed to genotype the MS strains isolated from the mothers, grandmothers, and children.

- The AP-PCR amplification solution contained the following in a total volume of 50 µL.
  - 5 µL of 10X PCR reaction buffer
  - 200 µM of dNTP
  - 4.0 mM of MgCl₂
  - 2.5 units of Taq DNA polymerase (Invitrogen, U.S.A.)
  - 0.5 µM of primer (OPA-5, sequence: 5’-AGGGGTCTTG-3’, OR OPA-13, sequence: 5’- CAGCACCCAC-3’ (Gibco BRL, U.S.A.)
  - DNA template (FTA punch containing MS)

The amplification process was carried out for 35 cycles; each cycle consisted of 2 min at 94°C to denature the template DNA, 1 minute at 36°C to anneal the primer to template, 2 minutes at 72°C for primer extension, followed by 5 minutes at 72°C for extension of incomplete amplification. This AP-PCR process was similar to the previously published method by Saarela et al. (1996).
Fifteen µL of DNA from the AP-PCR product was separated by electrophoresis through a 1.0 % agarose gel using a Mupid-2 Mini Gel Migration System (Cosmo Bio Co. LTD, Japan) for 25 minutes at 100V. A 1 Kb Plus DNA Ladder (Invitrogen, U.S.A.) was used as a standard. Ethidium bromide stained gels were visualized with ultraviolet light and images stored with a digital photo documentation system (Fisher Biotech FB-PD-34). The electrophoretic DNA fragment patterns of each isolate were then compared to determine the genotype of the strains.

The transmission (source) of MS isolates from each subject was evaluated by comparing the AP-PCR DNA fragment patterns of MS isolates from each triad. AP-PCR products with OPA -5 and OPA-13 were compared for all isolates from the mother, grandmother, and child. Isolates with identical OPA-5 and OPA-13 AP-PCR DNA fragment patterns were considered identical genotypes (or strains).

3.4. DATA ANALYSIS

In brief, descriptive statistics assessed the distribution of the various measurements (i.e. age, DMFS/dmfs, MS per ml of saliva or swab, MS genotypes per individual, etc.) for the appropriate generational groups (grandmother, mother, and child). DMFS/dmfs indices and log10 MS levels were compared among the 3 generations with the paired t-test using the GraphPad Software. Bacterial culture data were analyzed with parametric statistical tests.

4. RESULTS

4.1 Subject Demographics
Ten grandmother-mother-child triads were recruited for this study. All mothers stated that they were the primary care-givers to their children. All grandmothers were from the maternal side of the family, and stated that they provided ≥20 hours of childcare per week. Mean ages, DMFS/dmfs, and MS counts are given in Table 1. All grandmothers and mothers except one grandmother had MS infection. The triad with zero MS for grandmother was excluded for analysis for MS transmission in the study. There were no significant differences in log10 MS CFU/mL of saliva (Mean±SD: 4.4±1.6, 4.6±1.0, 4.8±1.0, for children, mothers, and grandmothers, respectively). There were significant differences (paired t test, \( P \leq 0.05 \)) in DMFS/dmfs scores between grandmother-mother, mother-child, and grandmother-child (DMFS/dmfs Mean±SD: grandmother - 55.3±21.8, mother - 15.4±8.3, child - 33.4±16.3). There were 8 grandmother-mother–male child triads and 2 grandmother-mother–female child triads. Six Hispanic, 2 Asian (1 Chinese, 1 Vietnamese), 1 African American, and 1 African American/Caucasian families participated. All children had active caries, and all subjects were infected with MS except for one grandmother.

### 4.2 MS Transmission and AP-PCR

A total of 284 isolates were collected and fermentation test results identified all of them as MS. Two hundred fifty nine isolates were identified as *S. mutans* because of their ability to ferment mannitol, sorbitol, raffinose and melibiose. Twenty five isolates were identified as *S. sobrinus* because of their ability to ferment only mannitol and sorbitol.

AP-PCR with primer OPA-5 was performed on all MS isolates. OPA-13 was used only on isolates that showed the same AP-PCR patterns with OPA-5. The number of different strains in each subject was determined by comparison of the AP-PCR patterns.
of isolates from each subject with the two different primers. AP-PCR identified a total of 31 MS genotypes in the 9 grandmothers (29 *S. mutans* and 2 *S. sobrinus* genotypes). Twenty-eight genotypes were identified in the 10 mothers (27 *S. mutans* and 1 *S. sobrinus* genotypes). Finally, 22 MS genotypes were identified in the 10 children (21 *S. mutans* and 1 *S. sobrinus* genotypes). Figure 1 illustrates a family/triad that shows transmission between the grandmother and child, as well as another shared genotype between grandmother, mother, and child. The OPA-5 primer was used first (Figure 1a), and these results were confirmed using the OPA-13 primer (Figure 1b).

Four groups showed no transmission. Figure 2 demonstrates different groups that did not have any shared genotypes between subjects. The OPA-5 gel for groups 3 and 6 shows that grandmother, mother, and child all have different genotypes present (Figure 2a). For group 1, mother and child appear to have one shared genotype, in comparison to the grandmother. However, utilizing the OPA-13 primer clearly shows three distinct genotypes in all three subjects, illustrating the importance of using two primers.

The mean number ± SD of genotypes of MS was 3.4±1.7 for the grandmothers, 2.8±0.8 for the mothers, and 2.2±1.2 for the children. Of the 22 MS genotypes identified in the child, only 8 genotypes were transmitted from either the grandmother or mother. Out of these 8 genotypes shared, 4 genotypes were transmitted from the grandmothers, 3 genotypes were transmitted from the mothers, and 1 genotype was shared between grandmother, mother, and child. Fourteen genotypes in the children had unidentified sources. Twenty-five genotypes from the mothers were not transmitted, as they were present in the mother, but not the child. All transmitted genotypes from the mothers were *S. mutans*. Twenty-six genotypes from the grandmother were not transmitted to the child.
Of the 4 strains that were transmitted directly from grandmother to child, one strain was *S. sobrinus* and three were *S. mutans*. All members of one grandmother-mother-child triad shared a common genotype. This genotype was identified as *S. mutans*. In terms of the different patterns of vertical transmission in the triads, 1 of 9 (11%) showed grandmother-mother-child transmission, 1 of 9 (11%) showed grandmother-mother transmission, 3 of 10 (30%) showed mother-child transmission, and 4 of 9 (44%) showed grandmother-child transmission. Three triads showed single MS strain transmission, whereas 3 other triads had multiple strain transmission within the families (triad 4, triad 8, and triad 10). AP-PCR results for all triads with some pattern of transmission are shown in Figure 3. The number of genotypes per subject, as well as the transmission patterns for all groups is listed under Table 2.

One of the two grandmother-mother-female child triads showed MS transmission. Five of the eight grandmother-mother-male child pairs showed transmission. Transmission was observed in five out of the six Hispanic families and both Asian families, but neither of the African American and African American/Caucasian families showed transmission (Table 3).

A summary of transmission rates, as well as transmission patterns is listed under Table 4.
Table 1. Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean Age± SD</th>
<th>Log_{10} MS counts/mL saliva</th>
<th>DMFS/dmfs (child)</th>
<th>Gender Male:Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grandmother (G)</td>
<td>53.3±10.6</td>
<td>4.8±1.0</td>
<td>55.3±21.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Mother (M)</td>
<td>29.1±6.5</td>
<td>4.6±1.0</td>
<td>15.4±8.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Child (C)</td>
<td>4.0±0.7</td>
<td>4.4±1.6</td>
<td>33.4±16.3</td>
<td>8:2</td>
</tr>
</tbody>
</table>

Table 2. Genotypes of MS for each subject and transmission patterns

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>M</th>
<th>C</th>
<th>MS Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Group 2</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Group 3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Group 4</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>G-M-C, G-C</td>
</tr>
<tr>
<td>Group 5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>G-C</td>
</tr>
<tr>
<td>Group 6</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Group 7</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>M-C</td>
</tr>
<tr>
<td>Group 8</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>G-C, M-C</td>
</tr>
<tr>
<td>Group 9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>M-C</td>
</tr>
<tr>
<td>Group 10</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>G-C, G-M</td>
</tr>
<tr>
<td>TOTAL</td>
<td>31</td>
<td>28</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Subject Ethnicity and Transmission Rate

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th># of Triads</th>
<th># with Transmission (any pattern)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>African American</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Summary of patterns of transmission reported and rates in this study

<table>
<thead>
<tr>
<th>Transmission Pattern</th>
<th>#/total groups</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-M-C</td>
<td>1/9</td>
<td>11%</td>
</tr>
<tr>
<td>G-M</td>
<td>1/9</td>
<td>11%</td>
</tr>
<tr>
<td>M-C</td>
<td>3/10</td>
<td>30%</td>
</tr>
<tr>
<td>G-C</td>
<td>4/9</td>
<td>44%</td>
</tr>
</tbody>
</table>
Figure 1.
A) AP-PCR gels: Example of one triad with MS strain transmission, using OPA-5 primer. Lanes 1 to 10 show isolates from the child, lanes 11 to 20 are isolates from the mother, and lanes 21 to 30 are isolates from the grandmother. (*) indicates a shared genotype between grandmother and child (transmission). (◊) indicates another uniquely shared genotype between grandmother, mother, and child.

B) This is the same triad as shown in Figure 1A, but run with the OPA-13 primer. The same transmitted strains are labeled as above.
Figure 2.

A) OPA-5 gel showing three triads without transmission. Groups 3 and 6 both show that grandmother (G), mother (M), and child (C) all have different genotypes. In group 1, mother and child appear to have a shared genotype in comparison to grandmother.

B) Same samples as above using OPA-13. Note that mother and child from Group 1 now show different banding patterns and therefore do not have any shared genotypes according to OPA-13. This demonstrates the importance of utilizing two primers to verify different genotypes.
Figure 3. Groups with transmission of MS

A) This is a gel with samples run with OPA-5 primer. It demonstrates families that showed some type of transmission pattern. The solid yellow line indicates separation between different triads, and the dotted green line separates different forms of transmission within the same family.

B) These are the same samples as above, but run with OPA-13 primer. It confirms the transmission of MS.
5. DISCUSSION

As described in the literature review, previous reports of MS transmission have focused mainly on vertical transmission from mother to child. The reports range from ~40% in a study by Tan (2007) to ~88% in Brazilian children (Klein, Florio et al. 2004). To the author’s knowledge, no previous study has reported grandmother to child transmission of MS. The results of this study indicate multi-generational transmission of MS in families of children with early childhood caries, including grandmother-mother-child and grandmother-child transmission. One triad had a transmission pattern where the grandmother, mother, and child all shared one common genotype. In other published studies, this transmission pattern would have only been reported as a mother to child transmission, which would make the mother-child transmission rate in this study as 40%. This falls within the previously reported transmission rates.

Many previous studies have reported mother to child transmission rates as high as 88% (Klein, Florio et al. 2004), whereas more recent studies have reported lower rates. These lower transmission rates may reflect the changing dynamics of American households. Previously, mothers would be the primary caregivers of the children, staying at home while the fathers worked. However, due to changes in the economy and culture, it is becoming more common for mothers to work and find alternative ways for childcare.

In this study, we concluded that 4 of 9 (44%) triads showed grandmother to child transmission of MS. However, since this study represents a “snapshot” in time of the subjects’ oral microflora, it is a possibility that the grandmother passed the strain to the mother, the mother passed the strain to the child, and over time, the mother lost this
particular strain. However, due to the fact that the grandmother still possesses the strain, it is more likely that direct vertical transmission from grandmother to child occurred.

Previous studies report that when mothers harbor greater than \(10^5\) colony forming units (CFU) of MS per mL of saliva, the frequency of infecting their child was 58\%, compared to 6\% when mothers harbored \(10^3\) CFU of MS per mL of saliva (Berkowitz and Jordan 1975). All grandmothers and mothers recruited in this study demonstrated high DMFS scores. Five out of 10 mothers in this study had counts greater than \(10^5\) CFU of MS per mL of saliva. Of these 5 mothers, 4 had shared genotypes with their children. Three out of 9 grandmothers in this study had counts greater than \(10^5\) CFU of MS per mL of saliva. Of these grandmothers, all 3 had shared genotypes with the children. In one triad (group 4), there were two types of transmission patterns observed: grandmother-mother-child and grandmother-child; therefore in this family, the grandmother had two distinct genotypes that were shared with the child.

In this study, it was noted that in the families with any type of transmission pattern, the children all had multiple genotypes of MS (see Table 2). In contrast, all of the children of the families with no transmission, from grandmother or mother, demonstrated only 1 genotype of MS.

While previous studies have shown that the mother is a common source of MS transmission to the child (Berkowitz and Jordan 1975; Berkowitz and Jones 1985; Li and Caufield 1995), other studies have also reported fathers as a potential source of MS (Li and Caufield 1995; Emanuelsson, Li et al. 1998). Beyond mothers and fathers, other close contacts may also be common sources of MS to the children.
Horizontal transmission is defined as the transmission of bacteria between members of a group. As the child becomes older, they will likely be exposed to potential MS transmission in a horizontal manner. For example, siblings, extended family, peers, and the introduction of day-care centers/nurseries may all provide potential sources of MS for these children. The children enrolled in this study were between the ages of 3 and 6 years. This age group is likely to have started school or interact with peers of the same age. The results from this study show that approximately 64% of MS genotypes found in the children were neither from the mother nor the grandmother, indicating that horizontal transmission played an important role in the acquisition of MS genotypes by the children.

Ethnic differences in transmission rates have been noted in the literature. In one study, it was shown that MS from African American children were more likely to be genotypically matched to the mother in comparison to Caucasian children (Li and Caufield 1995). Another study demonstrated 44.7% fidelity in MS transmission between Chinese mothers and their children (Li, Wang et al. 2000). To the authors’ knowledge, no studies have been conducted to determine differences in transmission rates between Hispanic families and other ethnic groups. In this study, cultural differences were found in transmission rates. Both Asian families (one Chinese, one Vietnamese) and 5/6 (83.3%) Hispanic families demonstrated transmission of MS. According to our survey, the Vietnamese family reported that both mother and grandmother routinely shared utensils as well as pre-chewed food with the child. Neither the African American family nor the African American/Caucasian family in this study demonstrated transmission. While the sample size in this study is small, perhaps there are cultural and behavioral differences within families that need to be examined further to determine their role in
transmission. Factors such as feeding habits, particularly pre-chewing food and sharing utensils, can influence the transmission of MS. In addition, the participants in this study were primarily of Hispanic origin. Perhaps in this culture, it is more common to have more members of the extended family (e.g. grandparents, aunts, uncles, cousins, etc.) living under the same household. Because of this reason, these children may be colonized with MS from multiple sources and therefore, increase their risk of caries.

In this study, 14/22 (64%) of genotypes found in the child were from unknown sources. This suggests that the primary point to prevent MS transmission is at the level of the child, since MS strains may come from a variety of sources, both within and outside the family.

Methods of controlling MS transmission to children have been widely studied, including the use of chlorhexidine and antimicrobials by the caregiver, yet many of these studies have provided controversial results. The use of xylitol in gums and mints has shown promising results in the reduction of caries and transmission of MS from caregiver to child (Burt 2006; Thorild, Lindau et al. 2006). However, xylitol is more expensive than sorbitol (the standard sweetener in most sugar-free gums), and in order to achieve a therapeutic effect, one must consume between 6-10 grams of xylitol per day. This may not be the most cost-efficient way to prevent transmission of MS, particularly in the lower socioeconomic groups.

The American Academy of Pediatric Dentistry (AAPD) encourages parents and other caregivers to help each child establish a dental home by 1 year of age. The results from this study support the idea that earlier prevention, particularly at the level of the
child, may be necessary to prevent the high costs of restorative work and controlling the caries disease.

In this study, children in 44% of the groups were colonized by MS genotypes present in their grandmothers, but not their mothers. This indicates that grandmothers who provide childcare are a common source of MS transmission to their grandchildren. This supports the development of more broadly based family-oriented strategies to prevent the transmission of MS and the occurrence of ECC. Additionally, since the results of this study showed that 64% of the MS genotypes in the children came from neither the grandmother nor mother, the most important approach may be to focus prevention and intervention on the child.

6. **CONCLUSIONS**

- The results of this study population indicate multi-generational transmission of MS in ECC families
- Maternal grandmothers who provide childcare are a significant reservoir of MS, which may lead to the transmission of MS to their grandchildren
- More broadly based family-oriented strategies need to be developed to reduce MS transmission and therefore decrease the incidence of ECC
- Because 64% of MS came from other unknown sources, the most important intervention is likely to be at the level of the child
7. REFERENCES


APPENDIX I: Flyer Announcing Recruitment for Study

RECRUITING VOLUNTEERS!!!

A STUDY ON THE BACTERIA IN SALIVA THAT CAUSE CAVITIES

Who are we looking for?

- Children 3 - 6 years old who are patients of the UCSF Pediatric Dental Clinic
  AND
- Their mothers AND
- Their grandmothers who provide care for the children ≥ 20 hours a week

What will participants need to do?

- Mother, grandmother, and child will each provide a saliva sample for the study and receive a brief dental exam (about 15 minutes)

What will participants receive?

- A free dental examination
- $10 gift card each

For details, please leave your name and contact number with:

Dr. Joanne Rahman
UCSF Pediatric Dental Clinic
(415) 476-3276
APPENDIX II: Oral Survey/Questionnaire

UCSF School of Dentistry

Subject initial:

Child’s Name: ______________________________________________

First ______________________ Last ______________________

Child’s Birth date: __________ / __________ / __________.

Child’s gender: Male_______ Female_______

Telephone Number: ______________________

1) Are you the primary caregiver?
   □ Yes
   □ No

2) What is your age? ________________

3) Does the child have active caries (determined by exam)?
   □ Yes
   □ No

4) Has the child taken antibiotics or medications within the past 3 months?
   □ Yes, which ones? __________________________
   □ No

5) Is the mother generally in good health?
   □ Yes (Review of systems)
   □ No

6) Has the mother taken antibiotics or medications within the past 3 months?
   □ Yes, which ones? __________________________
   □ No

7) Does mother have periodontal disease?
   □ Yes
   □ No

8) How many hours a day does the mother spend caring for the child (not including sleeping hours)?
9) Does a grandmother provide childcare for the child?
   □ Yes
   □ No

10) If yes to 9), does the grandmother care for the child ≥20 hours per week?
    □ Yes
    □ No

11) What is the grandmother’s age? ________________

12) Is the grandmother the maternal or paternal grandmother?
    □ Maternal
    □ Paternal

13) Is the grandmother generally in good health?
    □ Yes (Review of systems)
    □ No

14) Has the grandmother taken antibiotics or medications within the past 3 months?
    □ Yes, which ones? __________________________
    □ No

15) Does the grandmother have periodontal disease?
    □ Yes
    □ No

**ORAL HYGIENE AND HABITS**

16) How often does the child get his/her teeth brushed?
    □ Not every day
    □ 1 time each day
    □ 2 times each day

17) Does the child use fluoride toothpaste?
    □ Yes, what kind? _____________________________
    □ No
    □ I do not know

18) How many times does the mother help the child floss his/her teeth in the past week?
    □ None
    □ One time
19) How many times a day does the child eat sweet things (i.e. soda, juice, sweets, etc.)?
   □ Never
   □ 1-3 times a day
   □ 4-6 times a day
   □ ≥6 times a day

20) Did the child see a dentist at least once a year in the past two years?
   □ Yes
   □ No

21) How often does the mother share food or utensils with the child?
   □ Daily
   □ Weekly
   □ Monthly
   □ Never

22) How often does the grandmother share food or utensils with the child?
   □ Daily
   □ Weekly
   □ Monthly
   □ Never

23) How often does the child share food or utensils with other people?
   □ Daily
   □ Weekly
   □ Monthly
   □ Never

24) Who does your child routinely share food or utensils with? Check all that apply:
   □ Father
   □ Siblings
   □ Babysitters
   □ Friends
   □ Others: ________________________

25) Was the child breast-fed?
   □ Yes, until what age? _____________
   □ No
APPENDIX III: Consent form

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Multi-generational colonization of cavity causing bacteria in families with young children with dental decay

This is a medical/dental research study. Your study collaborator(s), Joanne Rahman, D.D.S., and/or Charles I. Hoover, Ph.D. will explain this study to you.

Medical/dental research studies include only people who choose to take part. Take your time to make your decision about participating. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask your study collaborators.

You are being asked to take part in this study because you are the mother or grandmother of a child with dental caries, and grandmother provides at least 20 hours of childcare per week. A separate Permission Form is needed for participation of the child.

Why is this study being done?

The purpose of this study is to count the number of cavity-causing bacteria in children with dental cavities, their mothers, and their childcare-providing grandmothers. Shared bacterial strains will be identified.

• This study is funded by Division of Pediatric Dentistry and is part of Dr. Rahman’s training program for a specialty in Pediatric Dentistry. The study collaborators have no financial or proprietary interests in the outcome of this study.

How many people will take part in this study?

About 30 people (10 children, 10 mothers, & 10 grandmothers) will take part in this study.

What will happen if I take part in this research study?

If you agree to be in this study you will be asked to chew on a piece of paraffin wax to obtain a saliva sample. You will also receive a brief oral examination to determine your dental health. This will take approximately 15 minutes of your time. You will only be requested to submit one saliva sample and have one oral exam.
Can I stop being in the study?

Yes. You can decide to stop at any time. Just tell any of the study collaborators that you want to stop. Your decision not to participate will not affect the quality of dental health care you or the child receive in any way.

What side effects or risks can I expect from being in the study?

To our knowledge, there are no risks or discomforts associated with being in this study, beyond those experienced during a normal dental appointment. However, rare allergic reactions may occur in some individuals to various substances. If you suspect you are, or know you are, allergic to paraffin or paraffin-containing products you should decline to take part in this study.

Are there benefits to taking part in the study?

There will be no direct benefit to you from participating in this study. However, this study could help dentists learn more about the presence of cavity causing bacteria in the mouth and how they are transmitted. It is hoped that in the future this information will help reduce the incidence of tooth decay.

What other choices do I have if I do not take part in this study?

You are free to choose not to participate in the study. If you decide not to take part in this study, there will be no penalty to you. You will not lose any of your regular benefits, and you can still get your care from our institution the way you usually do.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record is kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your dental/medical records for research, quality assurance, and data analysis include:

- UCSF’s Committee on Human Research

What are the costs of taking part in this study?

You will not be charged for any of the study treatments or procedures, beyond the normal cost of treating the child’s dental decay.
Will I be paid for taking part in this study?

You each will receive a payment of $10 (in the form of a gift card) when all saliva samples have been collected and oral examinations completed from the child, the mother, and the grandmother.

What happens if I am injured because I took part in this study?

It is important that you tell your study collaborators, Dr. Joanne Rahman &/or Dr. Charles Hoover, if you feel that you have been injured because of taking part in this study. You can tell either of them in person or call them at (415) 502-1647 or (415) 502-2278.

**Treatment and Compensation for Injury:** If you are injured as a result of being in this study, treatment will be available. The costs of the treatment may be covered by the University of California, depending on a number of factors. The University and the study sponsor do not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at (415) 476-1814.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical/dental care. You can still get your medical/dental care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study collaborators Dr. Joanne Rahman at (415) 502-1647 or Dr. Charles Hoover at (415) 502-2278.

For questions about your rights while taking part in this study, call the office of the Committee on Human Research, UCSF's Institutional Review Board (a group of people who review the research to protect your rights) at 415-476-1814.
CONSENT

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.
You will be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you.
PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled.

If you wish to participate in this study, please sign below.

Date       Participant's Signature for Consent (mother or grandmother)

Date       Person Obtaining Consent
**APPENDIX IV: Data Collection Sheets**

**UNIVERSITY OF CALIFORNIA, SAN FRANCISCO SCHOOL OF DENTISTRY**

Multi-generational transmission of mutans streptococci study

**DMFS/dmfs Record Sheet**

<table>
<thead>
<tr>
<th>Subject’s initials:</th>
<th>Subject ID:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit Date: <strong><strong>/</strong></strong>/____</td>
<td>Mother ___ Grandmother ___ Child ___</td>
</tr>
</tbody>
</table>

**Charting:** Red = current decay, Blue = previous restorations, X = missing

![Chart Diagram]

**Comments:**

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
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[Signature]
Author Signature

[Date]
Date

6/9/09