

# Mutans Streptococci prevalence in Puerto Rican babies with cariogenic feeding behaviors

Lydia Lopez, DMD, MPH Robert J. Berkowitz, DDS Mark E. Moss, DDS, PhD Philip Weinstein, PhD

Dr. Lopez is associate professor, Department of Oral Diagnosis and Surgical Science, School of Dentistry, University of Puerto Rico; Dr. Berkowitz is professor, Department of Dentistry, School of Medicine and Dentistry, University in Rochester; Dr. Moss is assistant professor, Department of Community and Preventive Medicine, School of Medicine and Dentistry, University in Rochester; Dr. Weinstein is professor, Department of Public Health and Community Dentistry, University of Washington. Correspond with Dr. Berkowitz at robert\_berkowitz@URMC.Rochester.edu

#### Abstract

**Purpose:** Previous studies have demonstrated that babies are at higher risk for mutans streptococci (ms) colonization if their mothers have dense salivary ms reservoirs relative to babies who have mothers with negligible salivary reservoirs. This communication provides data that identifies another potential risk factor (use of a nursing bottle at bedtime and/or naptime that contains a substrate other than water) for baby infection by ms.

Methods: The study population consisted of 60 babies (28 males/32 females; mean age 15 mos; age range 12-18 mos) who were all healthy, caries free, and slept with a nursing bottle that contained a substrate other than water (NB+). Pooled maxillary incisor plaque and saliva samples were obtained and immediately placed in Reduced Transparent Fluid (RTF); they were serially diluted and plated onto Mitis Salivarius Agar plus Bacitracin (MSB) and blood agar plates within 4 hours of collection; the plates were incubated in an anaerobic environment for 48h at 37C and then placed for 24h under aerobiosis prior to examination; representative ms colonies were isolated and subjected to mannitol and sorbitol fermentation tests for taxonomic verification. Plates with colony counts between 20 and 300 were utilized to determine the % of ms in each sample.

**Results:** Fifty one of the 60(85%) babies harbored ms in at least 1 of the 2 samples. The 95% confidence interval for the proportion of subjects with detectable levels of ms was 73% - 93%. Fisher's exact test showed that babies 16–18 mos age were more likely to have detectable levels of ms than babies 12–15 mos age (p=0.01). Levels of ms in plaque and saliva were as follows: <0.1% (plaque 27/51, mean age 15 mos, sd 1.77; saliva 28/51, mean age 15 mos, sd 1.76); 0.1%-1.0% (plaque 4/51, mean age 14 mos, sd 1.5; saliva 6/51, mean age 15 mos, sd 1.46); >1.0% (plaque 14/51, mean age 16 mos, sd 2.1; saliva 11/51, mean age 16 mos, sd 1.91). The density of infection did not vary by age for plaque (P=0.32) or saliva (P=0.64).

**Conclusion:** These findings support the concept that NB+ is a strong indicator for ms infection in Puerto Rican babies; that prevalence of infection increases with age; and that density of infection does not vary with age in this population. (Pediatr Dent 22:299-301, 2000)

Previous studies have demonstrated that babies are at higher risk for mutans streptococci (ms) colonization if their mothers have dense salivary ms reservoirs relative to babies who have mothers with negligible salivary reservoirs.<sup>1,2</sup> This communication provides data that identifies another potential risk factor (use of a nursing bottle at bedtime and/or naptime that contains a substrate other than water) for baby infection by ms.

This paper uses baseline data that were collected in an ongoing clinical trial of preventive interventions for Puerto Rican babies at high risk for dental caries.<sup>3</sup> One of the inclusion criteria for this clinical trial required that the subject be using a nursing bottle that contained a substrate other than water. Substrates other than water included a broad range of liquids such as juices, chocolate milk, and sweetened preparations such as Kool-Aid. Findings from the baseline data set regarding prevalence of ms infection and relative density of ms infection in the study subjects are described.

#### Methods

**Study population:** The study population was comprised of 60 babies who were clients of a Women, Infants and Children (WIC) program in Puerto Rico. This population was composed of 32 females and 28 males with a mean age of 15 months (mos) and age range of 12-18 mos at their time of entry into the study. Inclusion criteria were as follows: (1) healthy child; (2) presence of 4 primary maxillary incisors (PMI) with no visible defects; (3) clinically caries free on inspection: (4) use of a bottle at naptime and/or bedtime that contained a liquid other than water (NB+). The University of Puerto Rico Institutional Review Board approved this human studies project.

**Bacteriologic procedures:** These methods have been described previously.<sup>3</sup> Pooled maxillary incisor plaque and saliva samples were obtained from each subject at their time of entry into the study. The plaque samples were obtained by swabbing the gingival 1/3 of the 4 PMI with a cotton swab; the saliva samples were obtained by saturating a cotton swab on the floor of the mouth for 15 seconds (s). The samples were immediately placed into vials containing 2 ml of Reduced Transport Fluid (RTF)<sup>4</sup> with glass beads and transported to the labora-

#### Table 1. Distribution of Mutans Prevalence By Age Among 60 Puerto Rican Babies Using Nursing Bottles That Contained a Substrate Other Than Water

Age Group	Number with Detectable Mutans (%)	Total
15 months and younge	er 28 (78)	37

Fisher's Exact Test P=0.01

 Table 2. Mutans Density in Plaque among Children

 with Detectable Mutans

Level	Number	Mean Age in Months	Standard Deviation
< 0.1%	27	15.14	1.77
0.1-1.0%	4	14.40	1.50
> 1.0%	14	15.85	2.10

F-test P=0.32

Table 3. Mutans Density in Saliva among Childrenwith Detectable Mutans					
Level	Number	Mean Age in Months	Standard Deviation		
< 0.1% 0.1-1.0% > 1.0%	28 6 11	15.25 14.80 15.63	1.76 1.46 1.91		

F-test P=0.64.

tory for processing within 4 hours (h) of collection. The samples were dispersed with a vortex mixer for 30 s at setting 6. Appropriate dilutions were made in RTF with a repeat dispersion on the vortex mixer for 10 s at setting 3. Plated onto Mitis Salivarius-Bacitracin (MSB)<sup>5</sup> and blood agar plates were 0.1 ml aliquots of undiluted and appropriately diluted suspensions. The plates were incubated at 37° C for 48 h under anaerobic conditions. Representative colonies with morphological characteristics of ms were isolated and biochemically verified to be ms utilizing mannitol and sorbitol fermentation tests.<sup>6,7</sup> Plates with colony counts between 20 and 300 were used to determine the total number of cultivable bacteria in the sample. The density of ms in each sample was expressed as a percentage of the total colony count on blood agar.

Data analysis: Fisher's exact test was used to compare the frequency of ms infection in 16-18 mos old subjects versus 12-15 mos old subjects. Density of ms infection as a function of age was assessed in plaque and saliva using analysis of variance. Density of infection was stratified into 3 groups: <0.1%; 0.1%-1.0%;>1.0%. Variability in the estimate of the proportion of subjects with detectable levels of ms was quantified using a 95% confidence interval.

### Results

Fifty-one of the 60 babies (85%) harbored ms in at least 1 of the 2 samples. The 95% confidence interval for the proportion of subjects with detectable ms levels was 73% - 93%. Table 1 shows the proportion of study subjects with detectable levels of ms by age in mos. Fisher's exact test showed that babies 16-18 mos age were more likely to have detectable levels of ms than babies 12-15 mos age (*P*=0.01).

Tables 2 and 3 show levels of ms in plaque and saliva, respectively, in subjects with detectable levels. The density of infection did not vary by age for plaque (P=0.32) or saliva (P=0.64).

#### Discussion

The observation that the prevalence of ms infection in the 16-18 mos old babies was greater than the 12-15 mos old babies (p=0.01) is consistent with earlier reports which indicate that the prevalence of ms infection increases with age.<sup>8</sup> The finding that there was no relationship between density of ms infection and age was unexpected. This may be the result of multiple factors that were not included in the study's design. For example, maternal education about oral health and hygiene behaviors may have an impact on the density of mutans and may also vary with the age of the child.

Earlier studies regarding the acquisition of ms in babies, harboring 6-8 primary incisors (approximately 14 mos age), report an infection rate of approximately 25%.<sup>8</sup> These studies did not report the presence or absence of the NB+ variable. The observation in this report that 85% (51/60) of NB+ babies aged 12-18 mos were infected with ms supports the concept that NB+ is a strongly associated with ms infection in babies. We hypothesize that the reason for the high prevalence of ms in this population is related to the use of a nursing bottle that contains a cariogenic substrate. This concept is consistent with animal studies which demonstrate that implantation of ms is enhanced by such substances<sup>9</sup> as well as work in 6-24 monthold children in a Connecticut WIC program that showed ms infection in 50% of babies that used a bottle containing a sweetened beverage.<sup>10</sup>

Mothers with dense salivary reservoirs of ms are at higher risk for infecting their babies relative to mothers with negligible ms salivary reservoirs.<sup>1,2</sup> Accordingly, it seems reasonable to speculate that mothers with dense ms infection who expose their babies to the NB+ variable represent a significant high risk group for early ms infection of their babies. Studies indicate that children who acquire ms prior to 2 years of age are at higher risk for future caries than children initially colonized by ms at an older age.<sup>11,12</sup> Collectively, these studies expand our knowledge regarding risk for early ms infection and underscore the need for dental evaluation during the first year of life.

#### Conclusion

- 1. These findings support the concept that NB+ is a strong indicator for ms infection in Puerto Rican babies.
- 2. The prevalence of infection increases with age
- 3. The density of infection does not vary with age in this population.

This study was supported by NIH grants R03 DE12053 and 1P20RR1126.

## References

- 1. Berkowitz RJ, Turner J, Green P: Maternal salivary levels of Streptococcus mutans and primary oral infection of infants. Arch Oral Biol 26:147-49, 1981.
- 2. Kohler B, Brathall D, Krasse B: Preventive measures in mothers influence the establishment of the bacterium Streptococcus mutans. Archs Oral Biol 28:225-31, 1983.
- 3. Lopez L, Berkowitz R, Zlotnik H, Moss M, Weinstein P: Topical antimicrobial therapy in the prevention of early childhood caries. Pediatr Dent 21:9-11, 1999.
- Syed SA, Loesche WJ: Survival of human dental plaque flora in various transport media. Appl Microbiol 24:638-44, 1972.
- 5. Gold OG, Jordan HV, van Houte JA: Selective medium for *Streptococcus mutans*. Arch Oral Biol 18:1357-64.
- 6. Jordan HV, Englander HR, Lim S: Potentially cariogenic streptococci in selected population groups in the western hemisphere. JADA 78:1331-35, 1969.

- 7. Shklair IL, Keene HJ: A biochemical scheme for the separation of the five varieties of Streptococcus mutans. Archs Oral Biol 19:1079-81, 1974.
- 8. Berkowitz R: Etiology of nursing caries: a microbiologic perspective. J Public Health Dent 56:51-54, 1996.
- 9. van Houte J: Experimental odontopathic infections effects of inoculation methods, dietary carbohydrates, and host age. In Tanzer JM (Ed): Animal Models in Cariology (special supplement to Microbiology Abstracts-Bacteriology), pp. 231-38, 1981.
- Mohan A, Morse DE, O'Sullivan DM, Tinanoff N: The relationship between bottle usage/content, age, and number of teeth with mutans streptococci colonization in 6-24-monthold children. Community Dent Oral Epidemiol 26:12-20, 1998.
- Alaluusua S, Renkonen OV: Streptococcus mutans establishment and dental caries experience in children from 2-4 years old. Scand J Dent Res 91:453-57, 1983.

## Abstract of the Scientific Literature

### ACETAMINOPHEN INTOXICATION DURING TREATMENT

This review article presents the issue of acetaminophen (APAP) toxicity with therapeutic intent. The clinical literature and relevant information concerning the basic pharmacology and toxicology of this widely used nonprescription drug is reviewed for the practicing pediatrician. For over two decades, pediatricians have been made aware of the potential risk associated with the acute ingestion of large single and/or multiple doses of APAP. Recently, the hepatotoxic potential associated with "therapeutic" APAP administration has been brought to the attention of the pediatric community. A "risk profile" is developed with regard to factors that may predispose infants and children to this iatrogenic form of toxicity so that the awareness of physicians and other caregivers (including parents) can be heightened and preventative education administered.

**Comments:** Among the limited armamentarium of medications prescribed by pediatric dentists is the analgesic APAP. This article presents guidelines for its safe use. Among those listed are: not to administer APAP for conditions other than fever or mild-moderate pain, do not exceed the age-appropriate dose or duration of therapy, caution in patients receiving concomitant therapy with other drugs capable of inducing hepatic microsomal enzymes (e.g. anticonvulsants), recognize the full toxic potential of APAP and its signs and symptoms. **AK** 

Address correspondence to: Gregory L Kearns, PharmD, Chief, Section of Pediatric Clinical Pharmacology and Experimental Therapeutics, Department of Pediatrics, The Children's Mercy Hospital, 2401 Gillham Road, Kansas City, Missouri, 64108

Acetaminophen intoxication during treatment:What you don't know can hurt you. Kearns GL, Leeder JS, Wasserman GS. Clin Pediatr. 39: 133-44, 2000

41 references