Streptococcus mutans Levels and Biotypes in Egyptian and Saudi Arabian Students During the First Months of Residency in the United States

M.M. FARGLHY, S. EKLUND, and W.J. LOESCHE

School of Dentistry, The University of Michigan, Ann Arbor, Michigan 48109

Several studies indicated that serotype/biotype c strains are the most common on a global basis, but that regional differences may occur relative to other serotype/biotypes. Of particular interest is the observation that individuals residing in the Middle East have higher incidences of serotype e and d strains relative to their levels in American citizens. This could reflect exposure to different Streptococcus mutans serotypes during the period in which the teeth are colonized, or might reflect other factors local to the region, such as diet.

The purpose of the present study was to observe Egyptian and Saudi Arabian students during the first four months after their arrival in the United States, in order to determine whether this change in habitat affected the levels and biotypes of S. mutans. The results of this study showed that biotype c strains were the most prevalent in saliva and plaque of these Egyptians and Saudi Arabian students, followed by biotype e and biotype d.

There was a drop in the number of S. mutans in the saliva and the proportions of S. mutans in the plaque after two months of residence in the United States, followed by a significant increase after four months of residence. This increase was most noticeable in subjects who had a higher number of decayed surfaces. In these subjects, the percentage of S. mutans in pooled occlusal plaque increased significantly, from 6.1 to 13.2%.


Introduction.

In the last 20 years, Streptococcus mutans has been identified as a potential human odontopathogen (Loesche, 1982). Strains of S. mutans have similar physiological characteristics (Carlsson, 1968; Drucker and Melville, 1971; Edwardsson, 1968) but are genetically (Coykendall, 1970, 1971, 1974) and serologically (Bratthall, 1970) heterogeneous. Shklair and Keene (1974, 1976) have developed a biochemical scheme for the separation of S. mutans into five biotypes, which correlate with the four genotypes and seven serotypes of S. mutans.

Bratthall (1972), using serotype-specific fluorescent antibodies, showed that S. mutans of serotypes c, d, and e have a global distribution. In Europe, Australia, and North America, serotypes c and d were the most predominant serotypes, whereas in Cairo (Egypt), serotypes a and b were predominant. In Japan, Hamada et al. (1976) showed that serotype c strains were the most prevalent in dental plaque of Japanese children. The d and e serotypes were rare, and serotypes a and b were not detected.

Qureshi et al. (1977), in a study of two groups of schoolchildren from New York and Toronto, utilizing the biotype scheme, found that the predominant biotype in both groups was biotype c, whereas biotypes e and d were frequently isolated. Bright et al. (1977), in a study of six-year-old children in Ohio, found that serotype c was the predominant serotype (79.7%), and that types e and f comprised 9.5 and 5.6%, respectively, of the isolated organisms.

Keene et al. (1977a), in a study of young naval personnel from Orlando (Florida), San Diego (California), and Saudi Arabia, and of Hawaiian children, found that biotypes a and b were rarely detected. The Saudi Arabian had an increased prevalence of multiple biotypes and a higher frequency of biotypes d and e, Keene et al. (1977b), in another study of 217 Saudi Arabian naval recruits visiting the United States, could not find biotypes a and b, whereas biotype c, alone or in combination with other biotypes, was found in 78% of the recruits. A control group of American recruits had biotype c as the most predominant biotype (97.0%). Significantly higher proportions of biotype e strains were noticed in the Saudi Arabians, occurring either alone or in combination with other biotypes. Those recruits who harbored higher percentages of biotype e strains had the highest DMFT score and the lowest number of caries-free individuals. Leone et al. (1982) found that serotype d was more predominant in Egyptians than in Americans.

These studies indicated that biotype c strains are the most common on a global basis, but that regional differences may occur relative to the other biotypes. Of particular interest is the observation that individuals residing in the Middle East have higher proportions of biotype e and d strains relative to levels found in American citizens, and may, in some cases, harbor biotype a and b strains, which are rarely encountered elsewhere in the world, except possibly in Tanzania (Kilian et al., 1980). This could reflect exposure to different S. mutans serotypes during the period in which the teeth are colonized in youth, or might reflect factors local to the region, such as diet.

The purpose of the present study was to observe Egyptian and Saudi Arabian students during the first four months after their arrival in the United States, in order to determine whether this change in habitat affected the levels and biotypes of S. mutans.

Materials and methods.

Sample selection. — Sixteen graduate students — 13 from Egypt and three from Saudi Arabia — were initiated into this study within a month of their arrival in the United States. They were adults ranging in age from 23 to 47 yr, and included five females and 11 males. Six Egyptians, who had resided in the United States for more than two years, were included in order to provide a long-term resident group for comparison purposes.

Data collection. — At the first sampling session, each participant was examined for dental caries using the DMFS index, as described by Radke (1972). The participants were questioned about any changes in their dietary habits since arrival in the United States, and the number of times per 24 hr that they ate the following items was recorded:
candy, soft drinks, cake, pie, cookies, fruit, pudding, Jello, sherbet, and sweet snacks. The long-term residents were asked the same dietary questions.

At the same session, a plaque sample was obtained from the central fissures of the upper and lower first permanent molars. If any one of the first permanent molars was carious or extracted, the adjacent second permanent molar or the second permanent pre-molar was utilized for sampling. A sterile hypodermic needle (gauge no. 26) held with a hemostat was used to sample the central groove three times from the distal to mesial. A separate needle was used to obtain the plaque samples from each of the four selected teeth. The four needles were dropped into one test tube containing 10 ml of reduced transport fluid (RTF) (Loesche and Syed, 1973). The participants were also requested to spit saliva into a wide-mouthed jar. A disposable pipet was used to take one milliliter of this saliva and to place it into a test tube containing 9 ml of RTF. Plaque and saliva samples were collected in a similar manner at subsequent visits. Prior to the final sampling session, the students were questioned for the second time about any changes that might have occurred in their dietary habits, and also about the amounts and types of sugars they consumed per day.

Laboratory procedures. — The RTF containing the saliva and plaque samples was dispersed by sonification for five sec, and was serially diluted in RTF. Appropriate dilutions were plated by means of a Spiral plater* (Loesche and Straffon, 1979) over a 2- to 4-log dilution range on MSB agar (Gold et al., 1973) and MM10 sucrose agar (Loesche and Straffon, 1979). The plates were incubated anaerobically for five days and were then examined using a dissecting microscope in order to enumerate characteristic S. mutans colonies and total colony counts. About five to ten representative S. mutans colonies were removed from an appropriate plate for each subject. These were subcultured in order to identify further the organisms according to their biotype (Shklair and Keene, 1976).

Thioglycolate without dextrose, containing 0.002% bromcresol purple, was used as the basal medium, and 0.5% mannitol, 0.5% mellibiose, 0.5% raffinose or 0.5% mannitol + 1% arginine was added aseptically to this basal medium. These media were dispensed into sterile screwcap tubes, inoculated with a series of test organisms, and read after 48 hr of aerobic incubation, by a change in color, i.e., positive = purple turning to yellow. The mannitol with arginine was used to differentiate between biotypes c and b.

Statistical analysis. — Both parametric and non-parametric statistical tests were utilized to test whether the levels and proportions of S. mutans, as well as the distribution of the biotypes, were different at baseline, second, and final sampling sessions. These same tests were used to compare the new arrivals with the long-term residents. The Michigan Interactive Data Analysis System (MIDAS) was employed to complete the required statistics.

Results.

The mean DMFT and DMFS of the newly-arrived students was 9.7 and 14.9, respectively, which was higher than the corresponding values in the long-term residents, i.e., DMFT of 3.3 and DMFS of 5.2. The mean age of both groups was about 32 yr.

The mean and median S. mutans levels in the saliva and its proportions in the occlusal plaques are summarized in Table 1. The median salivary level in the new arrivals was initially about 55,000 CFU per ml. These values decreased at the second-month sampling and then increased significantly at the fourth-month sampling (Table 1). The same directional changes over time were observed with regard to the proportions of S. mutans in the plaque samples. In both the saliva and plaque samples, the four-month value was significantly higher than the values obtained at previous visits (Table 1). The long-term residents were sampled only once, and their S. mutans values were not significantly different from those for the new arrivals.

The group of newly-arrived students was divided into a low- and high-decay group, using the group median value of three decayed surfaces as the partition value. The log total count of S. mutans in the saliva decreased in both groups at the two-month sampling interval, but then increased significantly at the four-month sampling interval (Table 2). The four-month value in the low-carious group was significantly higher than the initial value.

The percentage of S. mutans in the plaque samples of the low-carious group did not change significantly during the four-month period (Table 2). The percentage of S. mutans in this group was initially high, due to elevated proportions

* Spiral Systems, Inc., Cincinnati, OH

### TABLE 1

<table>
<thead>
<tr>
<th>S. mutans Parameters</th>
<th>New Arrivals (16)*</th>
<th>Long-term Residents (6)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 month</td>
<td>2 months</td>
</tr>
<tr>
<td>Log Total Count/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Median</td>
<td>4.7</td>
<td>4.6</td>
</tr>
<tr>
<td>% S. mutans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.8</td>
<td>2.6a</td>
</tr>
<tr>
<td>Median</td>
<td>1.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*No. of subjects in each group.

**Value in box is significantly different from other values in same row for new arrivals — mean values, paired t test, p < 0.05; median values, Wilcoxon rank test, p < 0.05.

**Values in same row are significantly different — paired t test, p < 0.05.

### TABLE 2

<table>
<thead>
<tr>
<th>Decay Surface Status</th>
<th>Mean Log Salivary Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean DS N</td>
<td>1 month</td>
</tr>
<tr>
<td>&lt;3</td>
<td>(0.3)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>(3.2)</td>
</tr>
<tr>
<td>Mean % S. mutans in Occlusal Fissure</td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>(0.3)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>(3.2)</td>
</tr>
</tbody>
</table>

*Value in box is significantly different from other values in row — Wilcoxon test, p < 0.05.

**Values with same superscript are significantly different — paired t test, p < 0.05.

***Significantly different using median test, p < 0.05.
of S. mutans in two persons. In the high-caries group, the percentage of S. mutans decreased at the second visit but then increased significantly to 13.2% at the third visit.

Biotype c strains were found in all subjects, at all visits, usually as the only biotype present (Table 3). In the new arrivals, biotype d strains were present in four of the plaque samples initially and in six samples at the four-month visit. Biotype e strains were present in the saliva of three subjects initially, but could not be detected at the four-month visit. Only one long-term resident harbored a biotype e strain.

No significant relationship was found between the amount and type of sugar consumed within a 24-hour period and the changes which occurred in the total count, log, and percentage of S. mutans in both saliva and plaque. Also, no statistically significant relationship was found which could associate a change in the dietary habits of the newly-arrived people with the total count, the log, and percentage of S. mutans in either saliva or plaque.

Discussion.

The results of this study showed that, among the 16 Arabic graduate students newly arrived in the United States, biotype c strains were the most prevalent, followed by biotypes d and e. Biotype a and b strains were not detected. These findings are in accord with the results of Keene and Shklair (1976, 1977), who found biotype c strains as the most frequently isolated biotype among Saudi Arabian naval recruits, but who could not detect biotypes a and b strains. These investigators noted that the Saudis had a significantly higher incidence of biotype e strains in their plaque samples, compared with those of United States naval recruits (Keene et al., 1977). Three of the new arrivals harbored biotype e strains in their saliva initially, but this biotype could not be detected at the four-month visit. Whether this indicates a real loss of this biotype, as a result of residence in the United States, cannot be determined from the present data.

The findings from the total counts of S. mutans in the saliva and proportions in the plaque, however, suggest that some re-arrangements of S. mutans occurred in the mouths of the new arrivals. Thus, there was a slight decline in the number and/or proportions of S. mutans in the second month after their arrival, followed by a significant increase in the fourth month. This pattern could reflect a response to environmental changes secondary to residence in the United States. A one-day diet history taken at the first and third visits did not reveal any differences in the intake of high-sucrose-containing food items. However, the dietary questionnaire employed may have been too insensitive to detect actual patterns of dietary changes over the period of residence in the United States.

This change in S. mutans values was also evaluated as a function of the initial number of decayed surfaces in the new arrivals. The salivary levels of S. mutans showed the same pattern of decline at two months, followed by an increase at four months for both the low- and high-decay groups. However, the plaque proportions of S. mutans only increased significantly at four months in the high-caries group (Table 2). This suggests that the initial number of carious lesions contributed to the observed pattern of change in the S. mutans values.

The caries scores of the newly-arrived Egyptians were higher than those of comparably-aged Egyptians who had previously emigrated to the United States. This could be attributed to a few individuals who had both a high caries score and high S. mutans levels. While these data are minimal, they do suggest that the incidence of S. mutans-associated caries may be increasing among Egyptian students.

Conclusions.

There was a drop in the number of S. mutans in saliva and a drop in the number and percentage in plaque after two months of residence in the United States, followed by an increase after four months of residence. This increase was most noticeable in subjects who had a high DMFS. In these subjects, the percentage of S. mutans in pooled occlusal plaque increased significantly, from 6.1 to 13.2%.

Acknowledgment.

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REFERENCES


HAMADA, S.; MASUDA, N.; OOSHINA, T.; SOBUE, S.; and


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**ANNOUNCEMENT AND CALL FOR ABSTRACTS**

A SECOND INTERNATIONAL CONFERENCE ON

"CLINICAL FACTORS AND MECHANISMS INFLUENCING BONE GROWTH"

University of California, Los Angeles, January 3-5, 1985

This interdisciplinary Conference will be a follow-up to the basic science conference* held in January, 1982, and will emphasize clinical science aspects of bone growth. A primary objective of the Conference is to update and integrate our understanding of the growth of bone with new knowledge and to offer directions for future clinical research. The content will cover factors and mechanisms which influence both normal and abnormal pre-natal and post-natal bone growth, both general and craniofacial, from the subcellular to the gross level. Clinical experimental approaches to bone growth will be emphasized, and limited attention will be given to basic science aspects. Titles and 100-200-word abstracts in English are solicited from various clinical and other biological disciplines including but not limited to: anthropology; computer science; genetics; metabolism; morphology; oral, maxillofacial and plastic surgery; orthopedics; pediatrics and pedodontics. Abstracts should include purpose, methods and materials, results, and conclusions. They should be submitted for consideration as soon as possible, but no later than June 30, 1984. Attendance will be limited. The proceedings of the Conference will be published.

Contact:

Andrew D. Dixon
Bernard G. Sarnat
Dental Research Institute
School of Dentistry
School of Medicine
Center for Health Sciences
University of California – Los Angeles (UCLA)
Los Angeles, CA 90024, USA


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**ANNOUNCEMENT**

National Allan A. Brewer Symposium

NEW CONCEPTS FOR INNOVATIVE FIXED PROSTHODONTICS

April 12-14, 1984

Hyatt Regency Orlando

Lake Buena Vista, Florida

Sponsored by: Eastman Prosthodontics Alumni Association and Eastman Dental Center, Rochester, New York

Honorary Symposium Chairman: Dr. Allan A. Brewer (first chairman, Department of Prosthodontics, Eastman Dental Center)

Chairman: Dr. Gerald N. Graser (present chairman, Department of Prosthodontics, Eastman Dental Center)

Program Director: Dr. John A. Oster (president, Eastman Prosthodontics Alumni Association)

Symposium is designed to benefit general dentists, prosthodontists, periodontists, orthodontists, dental laboratory technicians, commercial laboratory representatives, and others concerned with diagnosing or treating conditions requiring fixed prosthodontics. The concepts, methods, and materials characterizing modern fixed prosthodontics will be reviewed, the problems examined, and the physiologic and esthetic standards of adequacy discussed. Topics will include diagnosis and treatment planning and the need for coordination of all diagnostic, clinical, and laboratory phases; abutment preparation and impression procedures for fixed prosthodontics and consideration of the problems; articulation and articulators; the new, superior ceramo-metal alloys that have virtually replaced gold; the elements of esthetics—spatial, optical, and biologic considerations; the focus of research and the outlook for still further advancements in the near future.

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