

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

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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%.

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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. Shklar 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 Arabians 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 Radike (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:

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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% bromocresol 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-carries group was significantly higher than the initial value.

The percentage of *S. mutans* in the plaque samples of the low-carries 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

TABLE 1
S. MUTANS IN SALIVA AND PLAQUE OF EGYPTIAN STUDENTS.

<i>S. mutans</i> Parameters	New Arrivals (16)*			Long-term Residents (6)*
	< 1 month	2 months	4 months	
Saliva				
Log Total Count/ml				
Mean	4.8	4.4	5.4	4.8
Median	4.7	4.6	5.3	5.0
% <i>S. mutans</i>				
Plaque				
Mean	5.8	2.6 ^a	9.8 ^a	2.0
Median	1.3	1.2	3.8	2.6

*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.

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

TABLE 2
CHANGES IN *S. MUTANS* LEVELS IN NEW ARRIVALS AS A FUNCTION OF CARIES ACTIVITY.

Decay Surface Status	Mean Log Salivary Levels				
	Mean DS	N	1 month	2 months	4 months
< 3	(0.3)	6	4.7 ^b	4.2 ^a	5.3 ^{ab}
≥ 3	(3.2)	9	4.9	4.6 ^a	5.4 ^a
Mean % <i>S. mutans</i> in Occlusal Fissure					
< 3	(0.3)	6	5.4	1.7	4.8
≥ 3	(3.2)	9	6.1	3.2	13.2

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

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

^bSignificantly different using median test, *p* < 0.05.

*Spiral Systems, Inc., Cincinnati, OH

of *S. mutans* in two persons. In the high-carries 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

TABLE 3
BIOTYPES OF *S. MUTANS* IN SALIVAS AND PLAQUES
OF ARABIC STUDENTS; FREQUENCY DISTRIBUTION.

Biotype/ Serotype	New Arrivals			Long-term Residents
	< 1 month	2 months	4 months	
	Saliva			
I/c carriers	16 (3)*	15 (2)	15 (1)	6 (1)
IV/dg "	0	1 (1)	2 (1)	
V/e "	3 (3)	1 (1)	0	1 (1)
Not detected		1	0	
	Plaque			
I/c carriers	15 (4)	11	11 (2)	5
IV/dg "	4 (3)	1	6 (2)	0
V/e "	1 (1)	0	0	0
Not detected		4	1	1

*Multiple biotypes in single individual.

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-carries 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%.

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*Dixon, A.D. and Sarnat, B.G., Eds.: **Factors and Mechanisms Influencing Bone Growth**, New York: Alan R. Liss, Inc., 1982.

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